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An Experimental Determination of the Effect of Increasing the Minimum Age (or Size) of Fish at Capture upon the Yield per Recruit

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Majkowski, J., W. S. Hearn, and R. L. Sandland. 1984. An experimental determination of the effect of increasing the minimum age (or size) of fish at capture upon the yield per recruit. Can. J. Fish. Aquat. Sci. 41: 736–743.

The aim of this paper is to describe a tag release–recapture experiment designed to determine the quantitative effect of increasing the minimum age (or size) of fish at capture upon the yield per recruit for the current intensity of fishing. If the minimum age corresponding to the maximum catch weight per recruit for this fishing intensity is greater than the minimum age that currently applies in the fishery, this optimal minimum age can be determined from a single tag release–recapture experiment. The main advantage of the described tag release–recapture experiment over the experimental approach recommended in the past, but not yet applied, is a reduction in the use of trial and error procedures. Unlike the classical Beverton and Holt yield per recruit analysis, this approach does not require specific information on fish growth and natural and fishing mortalities. Consequently, it can be implemented for many complex fisheries systems. However, as with the classical approach, it does not enable useful predictions to be made when a change in the minimum age significantly alters the number of recruits to the fishable stock. The method of data analysis is developed and illustrated using data on southern bluefin tuna (*Thunnus maccoyii*).

On trouvera dans l'article qui suit la description d'une expérience de mise en liberté et de recapture de poissons marqués, menée dans le but de déterminer l'effet quantitatif, sur le rendement par recrue avec la présente intensité de pêche, d'une élévation de l'âge (ou de la taille) minimal des poissons à la capture. Si l'âge minimal, correspondant au poids maximal des prises à cette intensité de pêche, est supérieur à l'âge minimal actuellement en cours dans la pêcherie, on pourra déterminer l'âge minimal optimal à l'aide d'une seule expérience de marquage–recapture. Le principal avantage de cette expérience par rapport à l'approche expérimentale recommandée dans le passé mais non encore appliquée, est de réduire les tâtonnements. Contrairement à l'analyse classique du rendement par recrue de Beverton et Holt, il n'est pas nécessaire, pour cette méthode, d'avoir des données spécifiques sur la croissance et la mortalité naturelle et par pêche des poissons. On peut donc l'appliquer à plusieurs régimes halieutiques complexes. Cependant, comme dans le cas de l'approche classique, on ne peut obtenir d'utiles prédictions quand un changement d'âge minimal modifie de façon marquée le nombre de recrues ralliant le stock pêchable. Nous décrivons et illustrons cette analyse à l'aide de données sur le thon *Thunnus maccoyii*.

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One of the major objectives of fisheries studies is the development of methods for predicting the effects of different fishing strategies upon (1) the yield being derived from a fish population and (2) the biological state of that population. A fishing strategy can frequently be characterized by the minimum age (or size) of fish at capture and the intensity of fishing. Age and size are regarded in this paper as directly corresponding quantities. Therefore, we will not mention size further in the text.

Changes in the catch weight when the fishing strategy is altered are frequently predicted on the basis of the Beverton and Holt (1957) yield per recruit analysis or its modifications (e.g. see Lenarz et al. 1974; Goh 1977; Marten 1978; Acheson and Reidman 1982; Sainsbury 1984). The catch predictions based on the above-mentioned analyses are expressed per a fixed number of recruits to the fishable stock. Therefore, these predictions are useful only if the alterations in the fishing strategy do not change the number of recruits to the fishable stock (see discussion in Majkowski and Hampton 1983c). The Beverton and Holt (1957) yield per recruit analysis can be carried out only if accurate information on fish growth and natural and fishing mortalities is available. This information is frequently difficult or even impossible to obtain.

Allen (1953) proposed an alternative approach for qualitatively determining the minimum age of fish at capture that results in the maximum yield per recruit for the current fishing

intensity. He demonstrated that a given minimum age results in the maximum yield per recruit, if an infinitesimally small change in this age satisfies a certain condition. The parameters involved in this condition can be easily determined with the exception of one that he suggested can be estimated on the basis of a tag release–recapture experiment if fishing is not very intensive. If the fishing strategy is not optimal, the test also indicates whether the minimum age should be decreased or increased but not the magnitude of the change required. Hence, he suggested that the optimal minimum age can be determined by trial and error experiments. Because these experiments may involve numerous changes in the minimum age of fish at capture and intensive tagging programs associated with each change, the experiments may be logistically difficult to implement, time consuming, and costly.

The objective of this paper is to present a new method for quantitatively determining the effect of increasing the minimum age of fish at capture upon the yield per recruit for the current intensity of fishing. This determination is directly based on a single tag release–recapture experiment. If the optimal minimum age (i.e. that which results in the maximum catch weight per recruit for the current fishing intensity) is greater than the limit that currently applies in the fishery, the optimal minimum age can be determined directly without the trial and error experiments necessary in implementing Allen's (1953) method. This is one of the main advantages of the tag release–recapture experiment described in this paper and makes this determination a feasible alternative to the Beverton and Holt (1957) yield per recruit analysis.

The proposed approach to the yield per recruit analysis eliminates the necessity for accurate information on fish growth and natural and fishing mortalities. Consequently, it can be implemented for many complex fisheries systems. Like the Beverton and Holt (1957) approach, it does not enable useful predictions to be made when a change in the minimum age of fish at capture significantly alters the number of recruits to the fishable stock.

This paper describes how the tag release–recapture experiment required should be designed and how data derived from this experiment can be analysed. The method of data analysis is illustrated using southern bluefin tuna (*Thunnus maccoyii*) tag release–recapture data.

Tag Release–Recapture Experiment

Assume, as in the classical yield per recruit analysis, that fish spawning is an annual, discrete event. The age of recruitment to the fishable stock is usually chosen by biologists as the minimum age at which fish could be potentially caught in a commercial way. This age will be referred to as r. The fish at age r will be referred to in this paper as recruits.

A random sample of recruits should be tagged and released to the water. The geographical locations at which the fish are tagged should have the same spatial distribution as recruits. The tagged fish incidentally recaptured by taggers should be rereleased. The method of estimating the desired number of fish to be tagged in order to address the yield per recruit problem with a specified accuracy is outlined in Appendix C.

Ideally, fishermen should be instructed to rerelease all fish caught with attached tags. The rationale for the rerelease procedure will become clear in the next section. If the fishermen are unable to rerelease fish due to, for example, their poor condition caused by catching, it may be possible to tag and release fish equivalent to the recaptured fish as substitutes (they should have the same characteristics as the recaptured fish). This tagging operation should take place each time a tagged fish is caught and not rereleased. If this operation and fish rereleasing are not feasible, tag release–recapture data derived from the experiment outlined can be used to estimate the yield per recruit, but additional information on the rate of natural mortality has to be obtained (see the next section).

Reviews of the methods of tagging can be found in Begon (1979) and Jones (1979). The tagging method chosen should satisfy the following conditions: (a) tagged fish are not subject to death caused by tagging, (b) fishing and natural mortalities and migration rates are the same for tagged and untagged fish, (c) growth of tagged and untagged fish is the same, and (d) a tag is visible and its number is readable when a tagged fish is recaptured by a fisherman. It is advantageous to double-tag each fish because this increases the number of recaptures and allows us to estimate the rate of tag shedding (Kirkwood 1981). Records should be kept of the geographical position, date, tag numbers, and the weight (and length and age if possible) of each fish at the time of tagging. Similar information should be sought when a tagged fish is recaptured by a fisherman. This information can be collected and processed in the way described, for example, by Majkowski (1982b). All recaptures have to be reported to tagging authorities, or the rate of nonreporting has to be estimated.

Method of Data Analysis

The yield per recruit, Y_m/N, for the current fishing intensity and for all minimum ages (m) of fish at capture greater than the current minimum age may be determined from results of the tag release–recapture experiment described using the formula

$$(1) \qquad Y_m/N = \sum_{i=1}^{T} \delta_{mi} w_i/X = R_m/X$$

where T is the total number of recaptures including second or subsequent recaptures, $\delta_{mi} = 1$ if the recapture is the first one for that fish at age greater than m, otherwise $\delta_{mi} = 0$, w_i is the weight of the fish at the age at recapture, and X is the number of recruits tagged. A fish for example recaptured and released twice at ages m_1 and m_2 ($m_1 < m_2$, the corresponding weights are w_1 and w_2, respectively) contributes w_1 to R_m if $r \leq m \leq m_1$, $m_1 < m \leq m_2$, and does not contribute anything to R_m if $m_2 < m$. The validity of equation (1) derives from a deterministic, age-dependent model of the population and is further discussed in Appendix A. An expression for the standard error of R_m/X is given in Appendix C. It is based on a binomial distribution for the recaptures with probabilities determined by a deterministic population model.

If the weights at release or recapture were not recorded, they may be calculated on the basis of a weight–length relationship and appropriate lengths. If the lengths at recapture were also not recorded, these lengths may be estimated on the basis of a growth equation.

If tag shedding and/or nonreporting of recaptures occur, they should be accounted for when R_m is being calculated. Models of tag shedding are reviewed by Kirkwood (1981). Nonreporting of recaptures can be accounted for in a way similar to those described by Seber (1982) and Ricker (1975).

If there are any fish younger than m that have not been rereleased by fishermen (see the previous section), this will result in a value of R_m smaller than in the case with the ideal

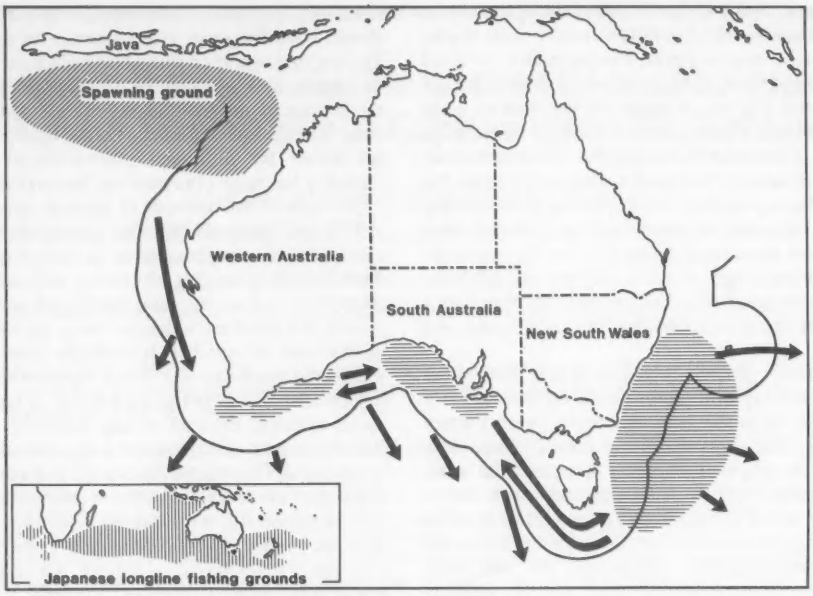

FIG. 1. Southern bluefin tuna spawning ground (diagonally hatched area), migration pattern off the Australian coast (arrows indicate the primary direction of fish migration and emigration from the 200-mile Australian Fishing Zone, the boundary of which is indicated by the solid line), and operation areas of the Australian fisheries (horizontally hatched areas) off the coast of Western Australia, South Australia, New South Wales, and the Japanese fishery (vertically hatched area).

experiment. To compensate for this bias, the number (X) of recruits tagged should be reduced (this reduced number will be referred to as X'). For each recaptured fish younger than m that should have been rereleased but has not been, X should be reduced by f_i^{-1} where f_i is the probability of survival of the tagged fish in the absence of any fishing up to the age at recapture,

$$t_i \left(\text{i.e. } f_i = \exp\left[-\int_r^{t_i} M(t')dt' \right] \right)$$

where M is the instantaneous rate of natural mortality. The rationale for this discounting procedure is discussed in Appendix B.

The value of f has to be estimated externally with respect to the tag release–recapture experiment (see the Example section), which is usually a difficult task. Therefore, the appropriate design and execution of a tagging experiment that eliminates the necessity of estimating f is very important. If the tagging experiment was not properly designed and/or implemented and the estimated f values are highly uncertain, the effect of this uncertainty upon the results of the analysis can be examined on the basis of sensitivity analysis (for methods see reviews in Majkowski 1982a, 1983).

Not only the yield from the entire fishery for different minimum ages of fish at capture may be of interest to fisheries biologists and managers, but also its specific fraction. For example, in the case of a multinational fishery, the yield per recruit values for a selected national segment may be required. In such a case, only weights of tagged fish recaptured by fishermen from this segment should be added to R_m. The tagged fish recaptured by these fishermen that were earlier recaptured and released by fishermen of the remaining segments should not be included in R_m.

The effect of stochastic environmental fluctuations on yield per recruit values may be assessed by repeating the experiment for several years. Then, yield per recruit values can be estimated for each cohort separately and cohort to cohort variability can be evaluated.

Example

The method of data analysis is illustrated using data on southern bluefin tuna (*Thunnus maccoyii*).

Background Information

Southern bluefin tuna is a highly migratory species. It spawns in the waters off the south coast of Java (see Fig. 1) during September–March. Juveniles migrate to the waters off the coast of Australia passing Western and South Australia (WA and SA, respectively) and New South Wales (NSW). The general direction of their movement within the 200-nautical-mile (370.4 km) Australian Fishing Zone (AFZ) is from west to east. However, some fish also move in the reverse direction.

The schools of juveniles within the AFZ support Australia's most important and valuable fin-fish fishery (see Fig. 1). The fishing methods used are pole and line, purse seining, and to a small extent, trolling. Southern bluefin tuna passing Australia gradually leave the nearshore fishing areas and become subject to Japanese longlining (see Fig. 1). Further information about this population and the associated fisheries can be found in Shingu (1978), Majkowski et al. (1981), Murphy and Majkowski (1981), Hampton et al. (1984), and Majkowski and

TABLE 1. Results of the empirical yield per recruit analysis for the entire Australian (A), WA, SA, and NSW southern bluefin tuna fisheries.

r (yr)	m (yr)	X	X'	Yield per recruit[a] (kg/recruit) for A	WA	SA	NSW
1–2	2	2685	2681	0.46±0.05	0.00	0.11±0.03	0.34±0.04
	3	2685	2588	0.25±0.05	0.00	0.11±0.03	0.14±0.04
	4	2685	2531	0.09±0.03	0.00	0.04±0.02	0.05±0.02
2–3	3	22714	22281	0.53±0.02	0.00	0.30±0.02	0.23±0.01
	4	22714	21644	0.29±0.02	0.00	0.21±0.02	0.07±0.01
	5	22714	21157	0.05±0.01	0.00	0.05±0.01	0.00
3–4	4	5083	4951	0.58±0.05	0.00	0.48±0.05	0.10±0.03
	5	5083	4781	0.20±0.04	0.00	0.12±0.03	0.08±0.02

[a]The value following the ± sign is the standard error of the estimated yield per recruit.

Hampton (1983c), but even our brief description indicates that the population and associated fisheries is a complex system.

Tagging Program

The Australian southern bluefin tuna tagging program was initiated in the late 1950's (for details see Hynd 1969; Murphy and Majkowski 1981; Majkowski 1982b, 1982c; Majkowski and Murphy 1983). Fish were caught (mainly by pole and line but also by trolling), tagged, and released in the fishing grounds off the coasts of WA, SA, and NSW. The number of fish tagged has been up to several thousands of fish per year. Since the tagging program was not designed specifically to address the yield per recruit problem, the conditions mentioned in the Tag Release–Recapture Experiment section were satisfied only in an approximate way. Fish recaptured by fishermen were very rarely rereleased.

Nearly all fish were double-tagged using durable and easily noticeable (see tagging condition d) standard tuna dart tags (Majkowski 1982b). Because fish caught by pole and line and tagged in that way recover from the adverse effects of tagging within a few weeks (W. S. Hearn, unpubl. data), these effects are unlikely to be drastic. Consequently, the tagging mortality, if present, is very small (see condition a). Direct observations of fish behavior during tagging confirm this (K. Williams, CSIRO Division of Fisheries Research, pers. comm.). Hence, it seems that the tagged and untagged fish are subject to similar fishing and natural mortalities, migration, and growth (see conditions b and c).

The degree of nonreporting of fish recaptured with attached tags is unknown. The nonreporting by Japanese fishermen may be quite significant. The reported number (303) of fish recaptured by these fishermen, which corresponds to 32 902 releases of tagged fish in the late 1950's and 1960's, is extremely small. Recaptures by Japanese fishermen have been frequently reported to the tagging program organizers with a considerable lag, up to 12 yr, from the actual recapture dates. This suggests that nonreporting of the Japanese recaptures may also be frequent. The number of fish recaptured by Australian fishermen that corresponds to the above-mentioned period of tagging is relatively large (1620), but this does not necessarily mean that the nonreporting by these fishermen was insignificant.

Data Analysis

To illustrate the proposed method of tag release–recapture data analysis, the yield per recruit values for each of the WA, SA, NSW, and entire Australian fisheries are calculated. The majority of releases of tagged fish took place during 1959–69 and only the data obtained for these releases are analyzed. All releases from this period are combined to make the results representative of the whole period. The fish caught for tagging by trolling are excluded from the analysis because these fish did not satisfy condition c (see Majkowski 1982b).

The ages of fish at tagging and recapture are estimated on the basis of their lengths at tagging, their periods between releases and recaptures, and the age–length relationship determined by Kirkwood (1983) for southern bluefin tuna (for further information on such a method of age determination see Majkowski and Hampton 1983a, 1983b). If the weight of a fish at release or recapture was not recorded, it was estimated on the basis of the weight–length relationship derived by Robins (1963) for southern bluefin tuna and the length at tagging or recapture, respectively. In cases where the length at recapture was also unknown, it is estimated on the basis of the length at tagging, the period between release and recapture, and the length–age relationship (Kirkwood 1983). A statistical model developed by Kirkwood (1981) is applied to account for tag shedding (for further information on such an application see Majkowski 1982c). The few fish for which the above-mentioned information was not available and could not be estimated were excluded from the analysis. The parameter f_i, which is required because the rereleasing procedure was not implemented, is estimated on the basis of the instantaneous rate of natural mortality, M, of 0.2/yr (Hayasi et al. 1969) and the period at liberty, $t_i - r(f_i = \exp(-M(t_i - r)))$.

Since the estimate of M is considerably uncertain, the effect of this uncertainty upon the results of the analysis is assessed on the basis of ordinary sensitivity analysis (for the method, see reviews in Majkowski 1982a, 1983). It is expected that the error in the estimate of M is not greater than ±50% of its estimate (Majkowski and Hampton 1983c). Accordingly, the calculations are repeated using the M value changed by ±50%.

Results

The results presented in Table 1 clearly indicate that any increase in the minimum age of fish at capture would not result in an increase in the total Australian yield per recruit for the fishing intensity during the 1960's. If the minimum age of fish at capture is increased from 2 to 3 yr, the total Australian yield per recruit would decrease from 0.46 to 0.25 kg (Table 1), a decrease of 46%. Similarly, if the minimum age is increased

from 3 to 4 yr and from 4 to 5 yr, the total Australian yield per recruit would decrease by 45–64% and by 66–83%, respectively, depending on the definition of recruits.

The same conclusion can be reached if the effect of increasing the minimum age of fish at capture from the SA and NSW fishing grounds is analyzed. If the minimum age of fish at capture is increased from 3 to 4 and 4 to 5 yr, the yield per recruit for the SA fishing grounds would decrease by 30–64% and by 75–76%, respectively, and by 64–70% and by 20–100%, respectively, for the NSW fishing grounds. The effect of increasing the minimum age of fish at capture upon the extremely small (prior to 1969) WA catches cannot be examined.

The differences between the values of X and X' in Table 1 are small. This is due to the low rates of natural and fishing mortalities (see the Data Analysis section). It is concluded on the basis of sensitivity tests (see the Data Analysis section) that the results presented in Table 1 are insensitive to ±50% changes in the estimate of M. As a result of these changes, the Y_m/N values from Table 1 were altered by the amounts −0.8 to 1.0%.

It should be noted that the southern bluefin tuna population and fishery were subject to considerable changes in the 1970's and 1980's (Hampton et al. 1984). Hence, the estimated Y_m/N values do not reflect the present situation. Regardless of the insensitivity of Y_m/N values to changes in the estimate of M, these values may also not closely reflect the yield per recruit values during the 1960's if the nonreporting by Australian fishermen was significant. As mentioned earlier, the purpose of presenting the analysis for southern bluefin tuna is to illustrate the new approach to the yield per recruit analysis rather than to formulate any conclusions concerning southern bluefin tuna.

Discussion

The empirical approach to the yield per recruit analysis requires the execution of an expensive tag release–recapture experiment and a few years to elapse before tag release–recapture data can be analyzed. However, tagging may also be required in the case of classical analysis to estimate the mortality and growth rates. This estimation also cannot be made before a few years have elapsed after tagging.

As demonstrated, the proper design of tagging experiments eliminates the necessity for any information on the rates of mortality and growth. This is the major advantage of the proposed empirical approach over the classical analysis. Rereleasing all recaptured fish reduces the number of tagged fish required to derive the information necessary for application of the empirical approach and, consequently, the cost of the tagging operation. Multiple recaptures of fish tagged may also provide more information on mortality (Sandland and Kirkwood 1981), migration, and growth (Shepherd and Hearn 1983). However, practical difficulties may occur in accurately and quickly measuring and weighing each tagged fish immediately after recapture, recording its tag numbers and all other information required (see the Tag Release–Recapture Experiment section) and, then, rereleasing the fish without any delay and in an unharmed condition. Therefore, alternative, though inferior ways of designing the tagging experiment and analyzing tag–recapture data are also proposed for the cases in which rereleasing tagged fish is not feasible. The real difficulties with the empirical approach may be the selection of a tagging method that will satisfy tagging conditions a–c, and the estimation of nonreporting rate, but these difficulties may also occur in the classical approach if the rates of mortalities and growth are estimated on the basis of tag release–recapture data.

In the empirical approach to the yield per recruit analysis, unlike the classical approach, it is not necessary to formulate a mathematical model. A model-free method is likely to be more robust over a class of possible mathematical assumptions.

The principles of the empirical approach are simple. They can be easily understood by most people involved in the fishing industry, even by those having virtually no mathematical and biological knowledge. Hence, the information derived on the basis of this approach is more likely to be generally accepted and cooperation in implementing the approach more easily obtained.

Acknowledgments

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Appendix A: A Mathematical Proof for equation (1): $Y_m/N = R_m/X$

Consider a year-class recruiting to a fishable stock at age r. Denote the abundance of this year class by $N(t)$ where t is age. This abundance is determined by

$$\text{(A1)} \quad \frac{dN(t)}{dt} = -N(t)[F(t) + M(t)]$$

where $F(t)$ and $M(t)$ are the instantaneous rates of fishing and natural mortalities, respectively. The solution of this equation can be presented in the form

$$\text{(A2)} \quad N(t) = N(r) \exp\left\{-\int_r^t [F(t') + M(t')]dt'\right\}.$$

The assumption of the minimum age of fish at capture equal to m is equivalent to the condition of $F(t) = 0$ for t between r and m. The yield (Y_m) from the year-class under consideration can be calculated in such a case as follows:

$$\text{(A3)} \quad Y_m = \int_m^\infty F(t')N(t')w(t')dt'$$

where $w(t)$ is the weight of fish at age t and $N(t)$ is determined by equation (A2). Hence,

$$\text{(A4)} \quad Y_m/N(r) = \int_m^\infty F(t')w(t') \times \exp\left\{-\int_m^{t'} [F(t'') + M(t'')]dt''\right\} dt'.$$

Equation (A4) is a generalized form of the Beverton and Holt (1957) yield per recruit formula. Note that the value $Y_m/N(r)$ is not dependent on the level of recruitment, $N(r)$.

Denote by $X(t)$ the abundance of tagged fish alive at age t but that does not include tagged fish that were previously recaptured and rereleased at age greater than m. This abundance is determined by

$$\text{(A5)} \quad \frac{dX(t)}{dt} = -X(t)[F(t) + M(t)],$$

the solution of which is

$$\text{(A6)} \quad X(t) = X(r) \exp\left\{-\int_r^t [F(t') + M(t')]dt'\right\}.$$

In the case of the ideal experiment (see the Tag Release–Recapture Experiment section) the biomass, R_m, of fish recaptured for the first time at age m or greater can be expressed as

$$\text{(A7)} \quad R_m = \int_m^\infty F(t')X(t')w(t')dt'$$

where $X(t)$ is determined by equation (A6) and $F(t) = 0$ for t smaller than m. Hence,

$$\text{(A8)} \quad R_m/X = \int_m^\infty F(t')w(t') \times \exp\left\{-\int_r^{t'} [F(t'') + M(t'')]dt''\right\} dt'.$$

The right-hand side of equation (A8) is equal to the right-hand side of equation (A4). Thus,

$$\text{(A9)} \quad Y_m/N = R_m/X.$$

If the yield per recruit for only a specific segment of the fishery is being considered, equation (A9) can be easily proven to be valid in the same way as that described above providing that $F(t)$ is defined as the instantaneous rate of fishing mortality associated with only that particular segment of the fishery and $M(t)$ is replaced in equations (A1)–(A9) by $M(t) + F_0(t)$ where $F_0(t)$ is the instantaneous rate of fishing mortality associated with the entire fishery with the exclusion of the above-mentioned segment.

Appendix B: A Mathematical Proof of the Non-rereleasing Discounting Procedure

If all recaptured fish younger than m are released by fishermen (i.e. if the instantaneous rate of fishing mortality operating upon the tagged fish younger than m is equal to zero), the number, $X(m)$, of alive tagged fish at age m can be calculated on the basis of equation (A6) as follows:

$$\text{(B1)} \quad X(m) = X(r) \exp\left[-\int_r^m M(t')dt'\right].$$

If the above-mentioned condition is not satisfied, the number, $X'(m)$, of alive tagged fish at age m can be estimated as follows:

$$\text{(B1)} \quad X(m) = X(r) \exp\left[-\int_r^m M(t')dt'\right].$$

If the above-mentioned condition is not satisfied, the number, $X'(m)$, of alive tagged fish at age m can be estimated as follows:

$$\text{(B2)}\quad X'(m) = X(r)\exp\left[-\int_r^m M(t')dt'\right] - \sum_i \exp\left[-\int_{t_i}^m M(t')dt'\right]$$

where t_i is the age at recapture and the summation is carried out over all recaptured fish younger than m that were not rereleased. The term

$$\exp\left[-\int_{t_i}^m M(t')dt'\right]$$

is the probability of fish death due to natural causes during the period $[t_i, m]$.

Using equation (B2) as an initial condition for differential equation (A5), the latter can be solved for t greater than m as follows:

$$\text{(B3)}\quad X'(t) = \left\{X(r)\exp\left[-\int_r^m M(t')dt'\right] - \sum_i \exp\left[-\int_{t_i}^m M(t')dt'\right]\right\} \times \exp\left\{-\int_m^t [F(t') + M(t')]dt'\right\}.$$

The biomass, R_m', of fish recaptured for the first time at age older than m can be then calculated on the basis of equation (A7) providing that $X(t)$ in that equation is replaced by $X'(t)$:

$$\text{(B4)}\quad R_m' = \int_m^\infty F(t)w(t)\left\{X(r)\exp\left[-\int_r^m M(t')dt'\right] - \sum_i \exp\left[-\int_{t_i}^m M(t')\right]dt'\right\} \times \exp\left\{-\int_m^t [F(t') + M(t')]dt'\right\}dt$$

$$= R_m - R_m \frac{\sum_i \exp\left[-\int_{t_i}^m M(t')dt'\right]}{X(r)\exp\left[-\int_r^m M(t')dt'\right]}$$

$$= R_m\left\{1 - \frac{\sum_i \exp\left[\int_r^m M(t')dt' - \int_{t_i}^m M(t')dt'\right]}{X(r)}\right\}$$

$$= R_m\left\{1 - \frac{\sum_i \exp\left[\int_r^{t_i} M(t')dt'\right]}{X(r)}\right\}$$

Hence,

$$\text{(B5)}\quad R_m = R_m' \Big/ \left\{1 - \frac{\sum_i \exp\left[\int_r^{t_i} M(t')dt'\right]}{X(r)}\right\}$$

and

$$\text{(B6)}\quad Y_m/N = R_m/X(r) = R_m'/X(r)\left\{1 - \frac{\sum_i \exp\left[\int_r^{t_i} M(t')dt'\right]}{X(r)}\right\}$$

$$= R_m' \Big/ \left\{X(r) - \sum_i \exp\left[\int_r^{t_i} M(t')dt'\right]\right\}$$

$$= R_m' \Big/ \left\{X(r) - \sum_i f_i^{-1}\right\}$$

$$= R_m'/X'(r)$$

where

$$\text{(B7)}\quad X'(r) = X(r) - \sum_i f_i^{-1}$$

as required.

Appendix C: Standard Error of R_m/X

The assumption underlying the standard error estimation is that the X recruits tagged have capture probabilities given by the deterministic model used in Appendix A. Let μ_m be the expected weight of a fish recaptured for the first time at age greater than m. Denote by σ_m^2 the variance of these recapture weights. Let p_m be the probability that a tagged fish contributes to R_m (i.e. is recaptured at least once at age greater than m).

The number (x_m) of tagged fish contributing to R_m is considered to be a binominally distributed random variable whose mean is Xp_m and variance $Xp_m(1 - p_m)$ (see Kendall and Stuart 1963). The variance of R_m is obtained using a well-known statistical equality (see Seber 1982, p. 9):

$$\text{(C1)}\quad \text{VAR}(R_m) = \underset{x_m}{\text{VAR}}[E(R_m|x_m)] + E[\underset{x_m}{\text{VAR}}(R_m|x_m)]$$

where VAR denotes the variance, E denotes the expectation or mean, and the vertical bars denote conditional distributions.

According to the notation introduced:

$$\text{(C2)}\quad E(R_m|x_m) = x_m\mu_m$$

$$\text{(C3)}\quad \text{VAR}[x_m\mu_m] = Xp_m(1 - p_m)\mu_m^2$$

$$\text{(C4)}\quad \text{VAR}(R_m|x_m) = x_m\sigma_m^2$$

$$\text{(C5)}\quad E[x_m\sigma_m^2] = Xp_m\sigma_m^2.$$

Substituting equations (C3) and (C5) into (C1) and dividing by X^2 gives

$$\text{(C6)}\quad \text{VAR}(R_m/X) = X^{-1}p_m[(1 - p_m)\mu_m^2 + \sigma_m^2].$$

In practice, the right-hand side of equation (C6) can be evaluated by substituting x_m/X for p_m and, denoting the x_m weights at first recapture after age m as $w_{1,m}, \ldots, w_{x_m,m}$ substituting

$$\text{(C7)}\quad x_m^{-1}\sum_{i=1}^{x_m} w_{i,m}$$

for μ_m and

$$\text{(C8)}\quad (x_m - 1)^{-1}\sum_{i=1}^{x_m}(w_{i,m} - \mu_m)^2$$

for σ_m^2.

A slight bias is introduced by this substitution because of the p_m^2 term in equation (C6). This bias is extremely small when the number of fish tagged is not very small (e.g. about 1% for 100 fish tagged) and may be safely ignored here. It may be necessary to take account of tag shedding or fish mortality due to the effects of tagging. In this case it is necessary to substitute $w_{i,m}/[1 - Q(t_i)]$ for $w_{i,m}$ in estimation formulae (1) and (C6)–(C8), where $Q(t_i)$ is the probability of a fish dying from

the effects of tagging or shedding all its tags within t_i time units from tagging (e.g. see Majkowski 1982c). The $w_{i,m}$ values are transformed as described above to represent the total weight of recaptures that would have been reported but for tag loss and tag-related death of fish. No attempt has been made here to take account of the uncertainty of $Q(t)$ in variance formulae (C6)–(C8).

It may be desirable to calculate the number of tagged fish, X, that results in a specific standard error (θ) of R_m/X. The specific value of X may be determined from the equation

$$\text{(C9)} \quad X = p_m[(1 - p_m)\mu_m^2 + \sigma_m^2]/\theta^2.$$

If such a calculation were to be performed to aid in the design of an experiment, it is necessary to know p_m, μ_m, and σ_m. As only plausible ranges of these quantities will be known before the experiment, it will be necessary to compute X for the extreme plausible values of the quantities. The number of fish to be tagged should be equal to the maximum calculated value of X.

Importance of Estuarine Residence for Adaptation of Chum Salmon (*Oncorhynchus keta*) Fry to Seawater

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Iwata, M., and S. Komatsu. 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. Can. J. Fish. Aquat. Sci. 41: 744–749.

Within 24 h after release from the Otsuchi Salmon Hatchery in Japan, most chum salmon (*Oncorhynchus keta*) fry migrated the 1.7 km and were found in the surface layer (10–15‰ salinity) of the estuary. No fish were seen in the underlying seawater. Many fry remained in the brackish water for 2 d before migrating seaward. Plasma Na concentrations increased gradually from 134 to 156 mmol/L during seaward migration from the river to Otsuchi Bay. When fry were acclimated to one-third seawater for 3 and 6 h and then transferred to seawater, the Na concentration of the fry increased maximally to 161–172 mmol/L within 12 h. When they were acclimated to one-third seawater for 12 h, the Na concentrations reached the seawater-acclimated level without showing any peak; subsequent exposure to seawater did not cause any further change in plasma Na. Acclimation to isotonic estuary water for 12 h is thus sufficient for efficient adaptation of chum salmon fry to seawater.

Dans les 24 h après mise en liberté à la salmoniculture d'Otsuchi, au Japon, la plupart des alevins de saumon keta (*Oncorhynchus keta*) émigrent en aval, et on les trouve dans la couche superficielle (salinité de 10–15‰) de l'estuaire. Aucune saumoneau ne se voit dans l'eau de mer sous-jacente. Plusieurs alevins séjournent en eau saumâtre pendant 2 jours avant de se diriger vers la mer. Les concentrations de Na plasmatique augmentent graduellement, passant de 134 à 156 mmol/L, au cours de la migration de la rivière à la baie Otsuchi. Chez des alevins acclimatés à un tiers d'eau de mer pendant 3 et 6 h, et ensuite transférés dans le l'eau de mer, la concentration de Na augmente pour atteindre un maximum de 161–172 mmol/L en moins de 12 h. Chez des alevins acclimatés à un tiers d'eau de mer pendant 12 h, les concentrations de Na atteignent le niveau d'acclimatation à l'eau de mer sans montrer de pic; l'exposition subséquente à l'eau de mer ne produit pas d'autre changement de Na plasmatique. L'acclimatation à une eau estuarienne isotonique pendant 12 h suffit donc à assurer une adaptation efficace des alevins de saumon keta à l'eau de mer.

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Chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon fry have strong preferences for seawater and migrate to the sea soon after yolk absorption (Hoar 1951; Houston 1957; McInerney 1964). They acquire salinity tolerance earlier than many other salmonids (Parry 1960; Conte and Wagner 1965; Conte et al. 1966; Weisbart 1968; Kashiwagi and Sato 1969). On direct transfer from freshwater to seawater, smaller fry were able to adapt to seawater more efficiently than larger fry. Osmoregulatory ability decreases gradually with increasing body weight or with time spent in freshwater (Iwata et al. 1982a, 1982b). Despite many experimental studies of seawater adaptation in salmonids (see review by Folmar and Dickhoff 1980), little information is available on adaptation during actual seaward migration in nature. Our objective was to observe the behavior of migrating chum fry in the river and the estuary and to assess the importance of estuarine residence time for seawater adaptation by acclimating fish first to one-third seawater for various periods before transferring them to full seawater.

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Materials and Methods

Underwater Observation and Field Sampling

Migrating fry were observed under water in the Otsuchi River from the release point at the Otsuchi Salmon Hatchery through the estuary to the bay (Fig. 1) during March and April from 1977 to 1981 with a scuba and diver propulsion vehicle (Farallon Industries, Belmont, CA). Fry were released from the hatchery at intervals of several days after most fry had migrated out from the river and the estuary. Net sampling was carried out during 1978–81. Fish were caught by square lift nets (1.8 × 1.8 m) in the river (station A) and at three stations (B, C, and D) in the estuary during the day of release and the next day (within 32 h after release). The number of migrating fry decreased rapidly 1 d after release. The net was placed on the river bottom or under the freshwater–seawater interface in the estuary for 5 min and pulled up quickly. Fry were also caught in the bay with the square lift net and a casting net in 1981. In this river, all chum fry are released from the Otsuchi Salmon Hatchery, and there is no natural spawning of chum.

Blood samples from 16 fish were collected at each point (stations A–G) on April 25, 1981, and immediately centrifuged

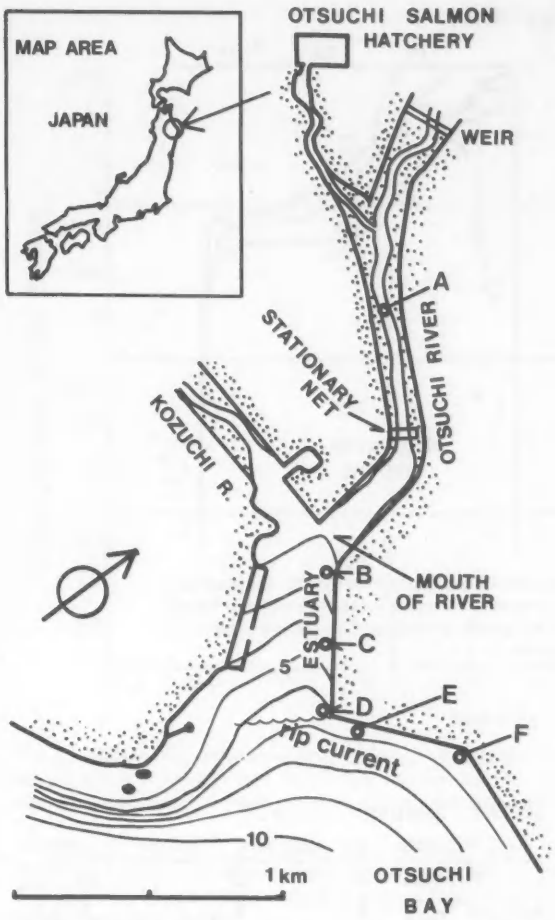

FIG. 1. Study area of the Otsuchi River and the estuary, Iwate, Japan. Ascending mature chum are caught by a stationary net from mid-November to mid-December. Eggs were transported to the Otsuchi Salmon Hatchery located 1.7 km upstream from the mouth of the river. A–F, sampling positions of migratory chum fry by nets. Underwater observations covered the area from the hatchery to the bay. Bottom contours show water depth (metres). Salinity profile in the estuary was monitored by a water quality meter from the river mouth to station E.

at 10 000 rpm; the plasma was stored on ice. The fish were also kept on ice and weighed in the laboratory within 2 h. Water temperature, salinity, and tidal level were recorded with a water quality monitor (Martek Instruments, Newport Beach, CA) at three stations in the estuary (B, C, and D). To measure salinity profiles, a boat was driven at constant speed with the monitor towed at depths of 0.2, 0.4, and 1.0 m.

Laboratory Experiments

Fertilized eggs from Otsuchi River chum salmon were transferred to the Otsuchi Salmon Hatchery and incubated at 10.0–11.5°C. Hatching occurred after 45 d and feeding was initiated 45 d after hatching. On March 23, 1981, two groups (1 and 2) of fry fertilized on December 18, 1980, and November 18, 1980, each consisting of about 1500 fish, were transferred to the Otsuchi Marine Research Center and reared at 10°C under natural photoperiod. Each group of fry was held in a 500-L tank and fed twice daily with artificial diet (Masu dry pellet Nos. 2 and 3, Nihon Nosan Kogyo, Tokyo) at the rate of 1.5% of body weight. To monitor the growth rate, we sampled 16 fry weekly; we calculated specific growth rates (SGR) of the two groups during 48 d, where $SGR = 100 \times (\log_e W_1 - \log_e W_0)/\text{day}$ and W_0 and W_1 are initial and final weights, respectively (Shelbourn 1980).

To determine the importance of estuarine residence on the ability of chum fry to regulate plasma Na concentrations, we transferred 16 chum fry with nets from the 500-L tanks to the 16-L aquaria. After 1 d in the 16-L test aquaria, one third of the water (freshwater) was replaced with seawater (10°C) by a pipe system; disturbance by transfer from one aquarium to another during the experiment was thus avoided. The change in the aquarium water from one-third seawater (12‰ salinity) to full-strength seawater (33.5‰ salinity) was carried out in the same manner. Salinity was determined by a salinity refrectometer (Atago, Tokyo) and a chloridometer (Buchler, Fort Lee, NJ).

Plasma Na concentrations were measured as described by Iwata et al. (1982a). Using Dunnett's procedure (Steel and Torrie 1960) we compared the experimental means with the control mean.

Results

Field Observations

The salinity gradient in the estuary is affected by tidal fluctuations, wind, water temperature, freshwater discharge, and currents in the bay. The river outflow forms an upper brackish layer (less than 0.5 m deep at low tide and 0.8–1 m at high tide) separated distinctly from the underlying seawater (32–33.5‰). The thin interface between the two layers, probably less than 10 cm, was clearly visible under water at high tide on a calm day. Vertical salinity profiles in the estuary are shown in Fig. 2. Freshwater from the Otsuchi River mixed rapidly with underlying seawater through the 800-m estuary. Longitudinal (800 m) salinity profiles adjacent to three sampling stations (B, C, and D) are shown in Fig. 3. At low tide, surface salinities ranged from 4 to 30‰ within less than 1 km, whereas at high tide the salinity of the same area was less than 15‰.

Observed under water on the first day, most fry were found in a restricted area (approximately 100 m long by 3 m wide) along the northeastern bank of the estuary, about 0.5 m deep. No fish were seen in the lower seawater layer or in midchannel away from the bank. Even when the fry were threatened from the surface with a net or stick, all stayed in the upper low-salinity layer. Fry appeared inactive on the first day and began feeding on the next day.

The total number of fry in the estuary for 2 d after release, as shown by net sampling, increased before high tide and decreased afterwards (Fig. 4). A maximum number of fish were caught during tidal phase II when there was virtually no water flow on the surface. No fish were observed to cross the rip current at the end of the estuary where the salinity was more than 30‰ (seaward off station D in Fig. 3), even though maximum concentrations of fry were observed at station D. Coincident with the falling tide on the day following release (phases III and IV), large numbers of fry were discharged into the bay with the surface water. This contrasted with behavior on the day of release when most fry at the end of the estuary returned to the

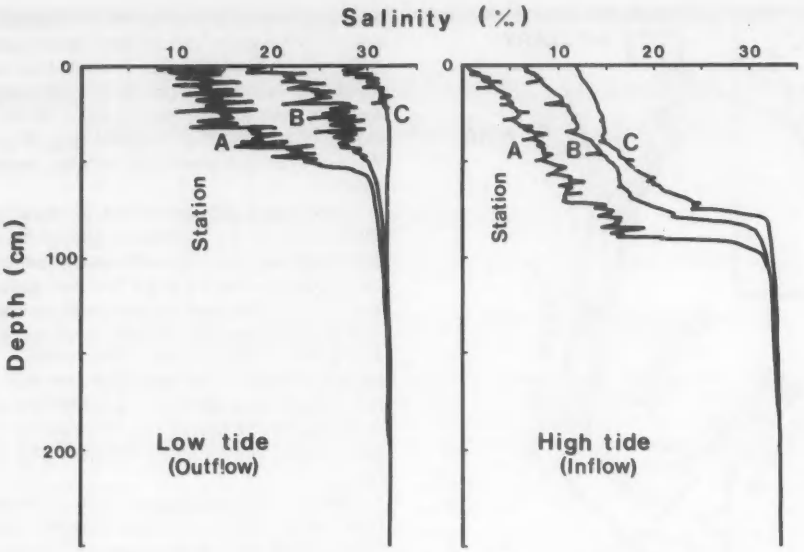

FIG. 2. Vertical salinity profiles in the estuary at stations B, C, and D at low (on the ebb, left panel) and high (on the flow, right panel) tide. Sharp salinity gradients, by which brackish water was clearly separated from the underlying seawater, were observed at high tide at depths of 0.8–1 m at stations B, C, and D. Fry were only observed in the upper brackish water.

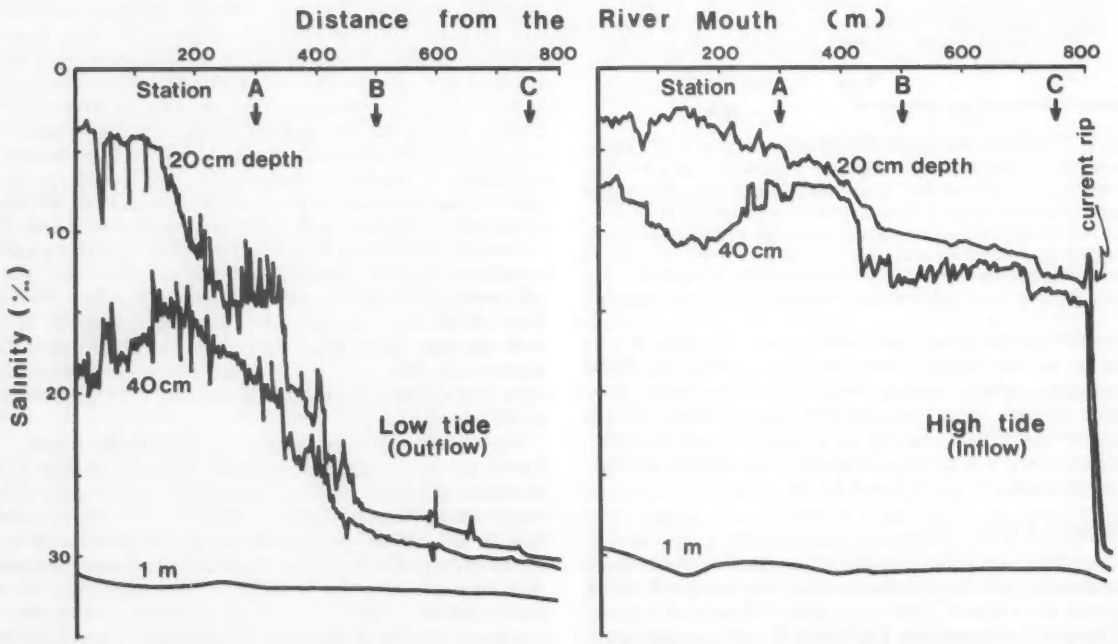

FIG. 3. Salinity profiles at low and high tide at depths of 0.2, 0.4, and 1 m throughout the estuary of the Otsuchi River. Salinity was measured by installing sensors of a water quality monitor (Martek) on a boat with an outboard motor and driving the boat at a constant speed. Fry were collected at stations B, C, and D.

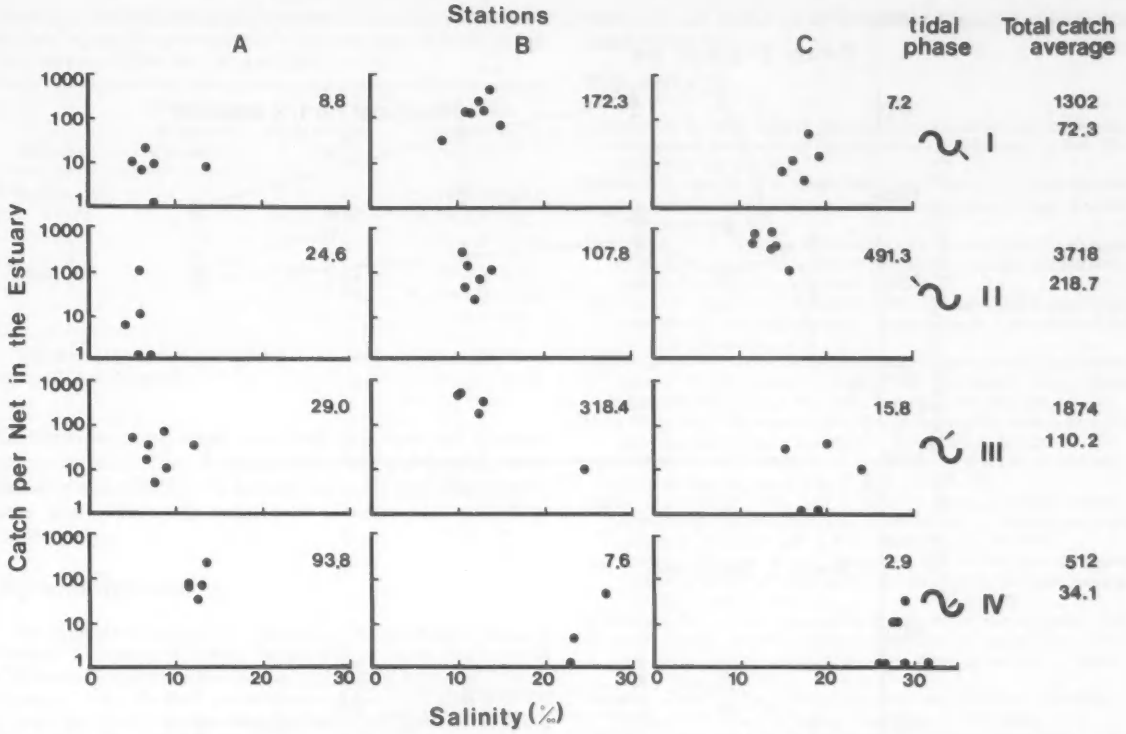

FIG. 4. Relationship between catch per net and salinity at stations B, C, and D in the estuary of the Otsuchi River. Tidal phases: I, inflow after low tide; II, inflow before high tide; III, outflow after high tide; IV, outflow before low tide. Fish were caught within 32 h after release. Numbers represent numbers of fry caught by square lift net; total catches and the means at each tidal phase are given at the right of the figure.

direction of station C after high tide. Although both the distribution of fry and salinity in the estuary shifted with tides, 90% of all fry caught were in salinities ranging from 10 to 15‰ (about isotonic to the fry).

Within 6 h of release, Plasma Na levels of the fish caught in the river increased from 134 ± 2.5 mmol/L within 6 h of release to 140–153 mmol/L, thus paralleling the increase in environmental salinity (Table 1). The plasma Na concentration of fry caught at station F on the day following release was almost the same as in fry caught at station G in the bay, which had remained in seawater for more than a week (Table 1).

TABLE 1. Salinity, mean (±SE) body weight, and mean (±SE) plasma Na concentration of chum salmon fry caught at various points in the Otsuchi River, estuary, and bay during seaward migration and within 32 h after release from the hatchery (see positions in Fig. 1) (N = 16).

Station	Salinity (‰)	Body weight (g)	Plasma Na (mmol/L)
A	—	0.74±0.06	133.9±2.5
B	9	0.66±0.06	140.4±2.8
C	14	0.63±0.05	145.0±2.0
D-1	19	0.91±0.05	145.6±1.5
D-2	20	0.80±0.06	149.1±2.0
E	26	0.79±0.04	155.7±2.1
F	29	0.79±0.04	156.6±1.8
G	33	0.94±0.07	156.1±1.6

Laboratory Experiments

The specific growth rates for the two groups of chum salmon fry during 48 d in laboratory aquaria at 10°C were 2.7 and 2.6%/d. Their mean ± SE (N= 16) initial and final body weights in grams were as follows:

Group 1	0.39±0.01	1.40±0.10
Group 2	1.51±0.09	5.14±0.35

The combined effects of size and prior exposure to one-third seawater on plasma Na levels in chum salmon exposed to full seawater are shown in Fig 5. Plasma Na concentrations of smaller fry and larger fry in freshwater were 138 ± 1.2 and 136 ± 2.6 mmol/L, respectively. After 3–12 h of acclimation in one-third seawater (time zero), mean plasma Na levels for smaller fry ranged from 146 to 150 mmol/L and for larger fry ranged from 145 to 154 mmol/L. With only 3 h of prior acclimation to one-third seawater, the plasma Na level of larger fry in seawater showed a peak 3 h after transfer to seawater and reached the seawater-acclimated level after 24 h (Table 2). The levels were significantly higher ($P < 0.05$ at 3 and 6 h) than for smaller fry. When they were maintained for 6 h in one-third seawater, the plasma Na level of larger fry increased after 12 h in seawater and reached the seawater-acclimated level. Plasma Na levels of smaller fry were regulated to less than 155 mmol/L during 24 h. With 12 h of prior exposure, the changes in plasma Na levels after all treatments ranged from 148 to 155 mmol/L after 24 h of exposure to full-strength seawater.

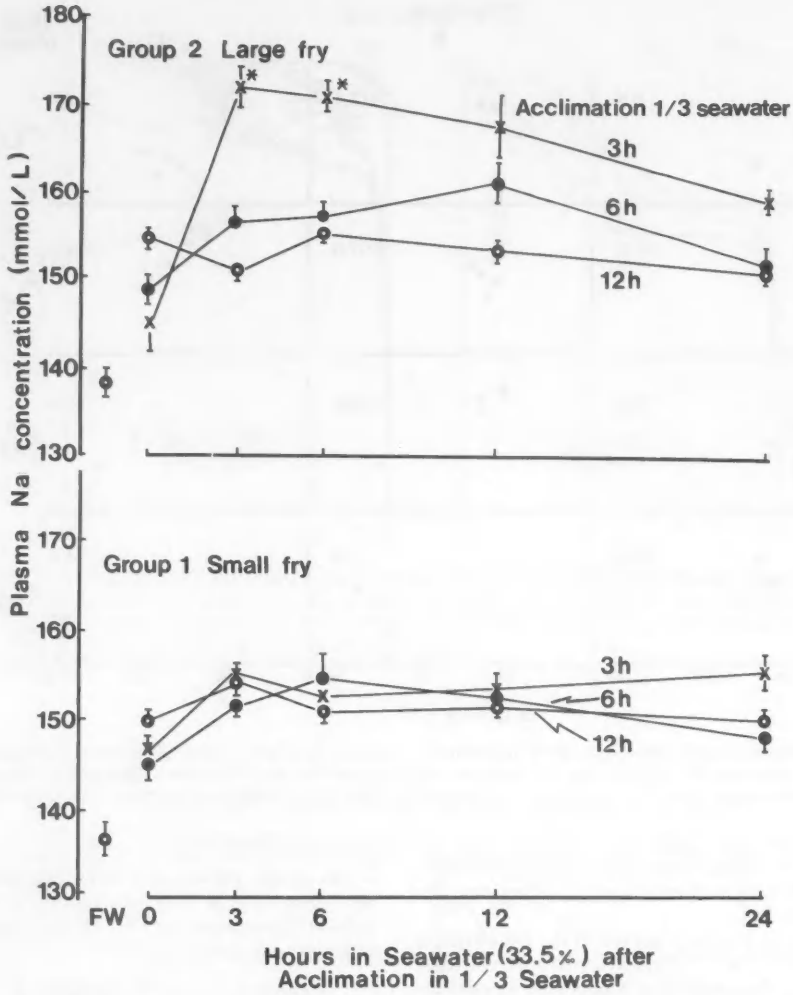

FIG. 5. Changes in plasma Na concentrations of two groups of fry in seawater (33.5‰) after acclimation for 3, 6, and 12 h in isotonic seawater (12‰). Upper panel, means ± SE for larger fry (mean body weight 1.9–2.1 g); bottom panel, means ± SE for small fry (0.75–0.80 g). FW = plasma Na levels of fry in freshwater.

Discussion

An earlier study (Iwata et al. 1982a) showed that smaller chum fry are better regulators of plasma Na than are larger fry during experimental transfer from freshwater to seawater (33.5‰). However, with larger fry in this study, prior exposure to one-third seawater (12‰) for several hours led to improved regulatory performance, similar to that of naturally migrating fry in the Otsuchi River. In that system, typical residence times in various parts of the system (intermediate and higher salinity areas) would allow for the physiological adjustments observed under experimental conditions. Migrating fry appear to prefer isotonic water; efficient adaptation in nature seems to depend on their initial acclimation to the isotonic environment of brackish water in the estuary. These observations are consistent with those of Houston (1957) and Baggerman (1960) on salinity preferences of chum fry and with Healey's (1979) estuarine residence times of 0–18 d for chum fry in a small British Columbia stream.

In recent years, the practice in Japanese hatcheries has been to rear chum fry until they reach a size of 1–3 g before release. Our findings suggest that rearing chum fry to a large size in freshwater may impair their ability to survive direct transfer to seawater. Our combined experimental and field results show the importance of residence in brackish water prior to movement into seawater. Thus, estuaries are an important area for the osmoregulatory adjustment of chum fry. Our findings may also apply to artificial seawater acclimation of chum fry and possibly to other salmonids before transfer into seawater net-pens or to the sea itself. This raises the possible utility of building salmon

TABLE 2. Maximum plasma Na concentration (±SE) during exposure of chum salmon fry to seawater (33.5‰) after prior exposure to isotonic seawater (12‰) for 3, 6, and 12 h (N = 16).

Group	Prior expose in isotonic seawater (h)	Body weight (g)	Maximum plasma Na (mmol/L)[a]
1 (smaller fry)	3	0.75±0.04	155.1±1.7 (12 h)[a]
	6	0.75±0.04	154.7±1.8 (6 h)
	12	0.80±0.03	152.9±0.9 (12 h)
2 (larger fry)	3	1.90±0.09	171.9±2.3 (3 h)
	6	1.99±0.10	161.1±1.9 (12 h)
	12	2.10±0.09	156.0±0.9 (6 h)

[a]Value in parentheses is the time after transfer to seawater when maximal level occurred.

hatcheries in coastal areas where both freshwater and seawater can be supplied. Use of one-third seawater and seawater after hatching and reducing the distance to be traveled downstream may also reduce the dangers of diesease and predation, respectively.

Acknowledgments

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Uptake of Zn, Cd, and Hg by Fish in the Presence of Competing Compartments

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Ramamoorthy, S., and K. Blumhagen. 1984. Uptake of Zn, Cd, and Hg by fish in the presence of competing compartments. Can. J. Fish. Aquat. Sci. 41: 750–756.

Rainbow trout (*Salmo gairdneri*) were exposed to Zn, Cd, and Hg in the presence of competing compartments compartmentalized in a novel experimental apparatus. In fish, uptake of Hg was more than doubled in the presence of Zn and Cd (0.45 μg/g). Organic matter decreased the uptake of Hg and Zn but enhanced that of Cd. We concluded that (1) equilibration with one heavy metal ion did not limit the fish in accumulating another heavy metal ion, and in addition to Hg uptake, 0.55 μg Cd/g and 22 μg Zn/g (wet weight) were taken up by fish, suggesting the possible existence of multiple binding sites in fish, (2) mass balancing showed excellent recoveries for Cd and Zn and losses up to 50% for Hg, and (3) the calculated partition coefficients (fish/substrate) for Zn, Cd, and Hg showed that these compartments are critical in the flux of Zn and Cd whereas sediment and suspended solids only determine the flux of Hg.

Des truites arc-en-ciel (*Salmo gairdneri*) ont été exposées à Zn, Cd et Hg en présence de compartiments concurrents, disposés dans un appareil expérimental nouveau genre. L'absorption de Hg dans les poissons fait plus que doubler en présence de Zn et Cd (0,45 μg/g). La présence de matière organique réduit la captation de Hg et Zn, mais favorise celle de Cd. Nous concluons que (1) l'équilibrage à l'aide d'un ion de métal lourd n'empêche pas les poissons d'accumuler un autre ion de métal lourd; les poissons, en plus de capter Hg, absorbent 0,55 μg Cd/g et 22 μg Zn/g (poids humide), ce qui donne à penser qu'il existe possiblement plusieurs sites de fixation chez les poissons, (2) l'équilibrage de masse indique d'excellentes récupérations pour Cd et Zn, et des pertes allant jusqu'à 50 % dans le cas de Hg et (3) les coefficients de séparation calculés (poissons/substrat) pour Zn, Cd et Hg indiquent que ces compartiments sont critiques dans le flux de Zn et Cd, tandis que seuls les sédiments et les solides en suspension déterminent le flux de Hg.

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Although several workers have reported on the accumulation by fish of Hg, Cd, and Zn separately (Kudo 1976; Prabhu and Hamdy 1977; Edgren and Notter 1980; Flos et al. 1979), studies on the uptake of multiple metals under conditions similar to natural waters are limited. In this study, we report uptake dynamics of Zn, Cd, and Hg by rainbow trout (*Salmo gairdneri*) in the presence of other environmentally significant transport processes. The reasons for choosing the above metals were as follows: (1) Zn, Cd, and Hg are group IIb metals with widely varying physicochemical properties, (2) Zn and Cd do not exhibit multiple valency whereas Hg can exist in 0, +1, and +2 valence states, (3) Zn resembles transition metals rather than Cd and Hg in its ability to form complexes with inorganic anions, (4) Cd^{2+} and Hg^{2+} are typical class "b" acceptors (Ahrland 1966; Pearson 1968a, 1968b) preferring S and Se donor atoms and form covalent compounds, whereas Zn^{2+} is intermediate between class "a" and "b" acceptors, (5) Zn can form complexes with both oxygen donors and S donor bases and is involved in the biosynthesis of nucleic acids and hence in tissue healing processes, and (6) Cd^{2+} and Zn^{2+} do not form stable methylated derivatives in the environment whereas Hg^{2+} undergoes biological and abiological methylation to form methylmercury and dimethylmercury, which are highly lipid soluble.

Since in the natural environment heavy metals (HM) are mostly distributed between sediment and water, we decided to follow the HM flux from sediment and water as the source and bioaccumulation under conditions similar to natural and polluted waters. In our study, we used a multicompartmental approach to investigate the intercompartmental interactions on the partitioning of HM.

Materials and Methods

Although HM exchange between the two-compartment water–sediment system has been studied, very little is known about HM exchanges in multicompartment systems. We used a simple laboratory simulation of an aquatic ecosystem to study the competitive intercompartmental interactions on HM uptake by fish. The compartments chosen were river water, suspended solids, sediment, cation- and anion-exchange resins (to represent the naturally occurring polyelectrolyte materials of plant origin whose ion-exchange strength and capacity vary from weak to strong depending on the composition, chain length, and functional group), and biota as represented by fish (rainbow trout). River water acted as the fluid transfer medium and the other compartments were separated by enclosing them in dialysis sacs and suspending them in the continuously stirred

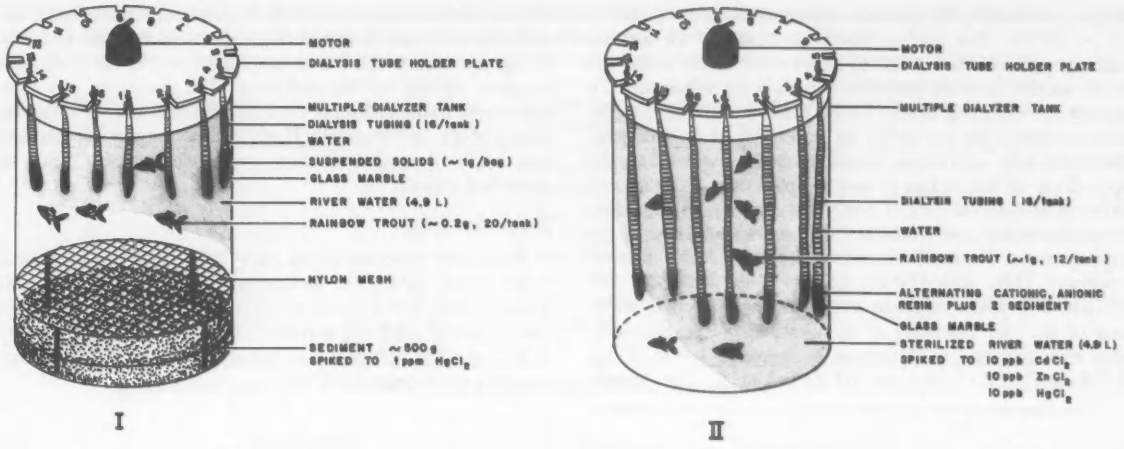

FIG. 1. Multicompartmental metal exposure system. (I) Spiked sediment (HM source) at the bottom of the dialysis tank; (II) river water (HM source).

water in a dialysis tank (Fig. 1). The dialysis tanks were kept in a temperature-controlled water bath at 15 ± 0.1°C. Illumination was provided by cool-white flourescent light, and incandescent bulbs simulated a 15-min dawn or dusk light intensity change. A constant 12-h light : 12-h dark photoperiod was controlled by an automatic timer.

Experiment I consisted of four dialysis tanks set up to follow the Hg flux from the sediment source (sediment spiked and equilibrated with 1 mg Hg/kg wet wt kept at the bottom of the dialysis tanks) (Fig. 1, I). The design of the four tanks was as follows: tank 1a = control, containing unsterilized sediment (500 g wet wt), 16 dialysis sacs each containing ~1 g dry wt of suspended solids (SS) and sterilized North Saskatchewan River (NSR) water (4.9 L); tank 1b = same as 1a plus twenty 8-wk-old rainbow trout (~0.2 g wet wt) each; tank 1c = same as 1b plus 10 mg dissolved organic matter (DOM)/L; tank 1d = same as 1b plus 30 mg DOM/L. At each sampling time, two fish and two dialysis bags containing SS and 200 mL of water were removed from the tanks and analyzed for Hg content.

Experiment II consisted of three dialysis tanks set up to follow the Hg flux from the water column as the source for Hg (Fig. 1, II). The experimental design of the tanks for experiment II was as follows: tank 2a = control, containing 4.9 L of sterilized tapwater spiked with 10 μg/L each of Zn, Cd, and Hg, 7 dialysis sacs each containing ~2 g wet wt of cation exchange resin, 7 dialysis sacs each containing ~2 g wet wt of anion exchange resin, 2 dialysis sacs each containing ~2 g wet wt of sediment; tank 2b = same as 1a plus 12 rainbow trout (~1 g wet wt each); tank 2c = same as 1b plus 10 mg DOM/L. At each sampling time, two fish, one dialysis sac containing cationic resin, one dialysis sac containing anionic resin, and 200 mL of water were removed and analyzed for Zn, Cd, and Hg content. One sac containing sediment was removed at 48 h and the other sac of sediment at 168 h for Zn, Cd, and Hg analysis.

Experiment II was designed to study the following: (1) HM flux from the source (water column), (2) uptake of Zn and Cd by fish and their effect on the accumulation of Hg, and (3) the effect of competing components, such as organic sediment and ion exchange resin (cationic and anionic) to simulate the synthetic and natural polyelectrolytes present in surface waters, on the uptake of Zn, Cd, and Hg by fish.

Experiment III was carried out as described below to determine the extent of photochemical methylation of Hg under conditions of this study. Inorganic Hg (10 μg/L) was added to two dialysis tanks containing 5 L of sterilized tapwater, two dialysis sacs of SS, one sac of cationic resin, and one sac of anionic resin in each tank. Tank 3a was exposed to light similar to experimental conditions and tank 3b was kept in dark covered with black cloth. Samples of water were withdrawn from the tanks at 0 h (actually 20 min), 6 h, 12 h, and 24 h and analyzed for total and inorganic Hg.

In a typical experiment, the dialysis sacs containing the chosen compartment(s) (SS, sediment, or ion-exchange resin) were suspended in 4.9 L of river water. The metals as analytically pure grade chlorides were added to the sediment in Experiment I or to the water column in Experiment II, and the fluxes of metal ions were followed in each compartment to determine the accumulation ratios of the compartments studied. The water was stirred with a magnetic stirrer and the sacs were rotated mechanically in a multiple dialyzer (Pope Scientific Co., U.S.). Dialysis sacs, whole fish, and samples of water were removed in duplicate at specified times and analyzed for total Zn, Cd, Hg, and methylmercury. Control values were obtained for background metal levels in all compartments and in the dialysis tubing. Wherever control levels were significant, necessary corrections were made in the calculations. Fish were not fed during the course of the experiment.

Biota

The first batch of fish used were 8-wk-old rainbow trout hatched from eggs in our laboratory (average wet wt 0.2 g). The second batch of fish were about 12 wk old (average wet wt 1.0 g). The average total Hg content in fish was 0.058 μg/g wet wt (whole fish), and 78% of it was methylmercury. The Cd and Zn concentrations were 0.11 and 15.0 μg/g wet wt, respectively.

Sediment

Sediment samples were obtained from the Vermilion River (a small stream flowing 0–42 m^3/s) approximately 5 km south of Vegreville, Alta. (53°27′N, 112°4′W) using an air-lift suction

sampler powered by the flow of compressed N_2 at 1.7×10^6 to 3.1×10^6 Pa. The surface layer (1–2 cm) of the bottom sediment was collected at 0.5- to 1.0-m water depths where the air-lift suction sampler performs at maximum efficiency. To regulate the sampling depth, a base plate was attached to the cover to support the unit gently on the surface of the sediment. This enabled the operator to maneuver the unit so that only the top 1–2 cm of bed sediment was sampled over a distance of 100 m at an interval of 1–1.5 m. The samples were pooled, mixed thoroughly, and stored at 2°C in contact with river water in an incubator. The bed sediment was a mixture of silt and sand (>250 μm (12), 250–150 μm (22), 150–63 μm (33), and <63 μm (33), where values in parentheses are percent distributions of the size fractions of the sediment). The bed sediment was analyzed to determine background levels of Hg (0.028 μg/g), Cd (0.2 μg/g), and Zn (48 μg/g). The organic content of the sediment was 12% dry wt). For Experiment I, the sediment was spiked with analytically pure mercuric chloride (1 mg/kg wet wt) and left to equilibrate at 2°C for 2 wk with frequent stirring of the sediment. The sediment was then transferred to the bottom of the dialyzer tanks and served as the source of Hg. In Experiment II, the sediment was not spiked and was enclosed in dialysis sacs (~2 g wet wt plus 5 mL of deionized water).

	Occurrence	CEC (mequiv./100 g)
Kaolinite	Podzols and tropical soils	3–15
Illite	Many soil types	10–40
Montmorillonite	Many soil types	95–150

Location of CEC	Surface area by water vapor method (m^2/g)	Area (nm^2/exchange site)
External	16.6	0.387
External	92.0	0.566
20% external and 80% in interlamellar spaces	750.0	1.545

The clay minerals suspended in water, which were analyzed for background levels of metals, will provide a larger surface area per gram than the sediment for interaction with HM and thus meaningfully represent the SS in rivers. The SS were placed in dialysis sacs, each containing 1 g dry wt plus 7 mL of deionized water. The average pore diameter of the dialysis sac membrane is 4.8 nm and the molecular weight cutoff is 12 000 (Fisher Scientific Ltd. 1982; Canadian Laboratory Supplies 1977). The dialysis sac is highly permeable to metals and their complexes (with organic ligands and fulvic acid) below molecular weight 12 000.

Ion-Exchange Resins

Cationic and anionic resins (Amberlite, IRC-50 (H) and IR-45 (OH), respectively) were swelled in water prior to placing in dialysis sacs (2 g wet wt plus 5 mL of deionized water in each sac).

River Water

NSR water (pH = 8.67 and conductivity = 375 μS) was used in Experiment I. Tapwater (treated NSR water) (pH = 8.44, conductivity = 295 μS) was used in Experiment II. Sterilized water (4.9 L) was added to the dialyzer tank as the fluid medium. The initial trace metal concentrations (milligrams per litre) for NSR water were as follows: Hg (<0.001), Cu (<0.001), Cd (<0.001), Zn (0.025), Co (<0.001), Pb (0.005), Ni (0.013), and Mn (<0.008).

Suspended Solids

Pulverized montmorillonite clay was used as suspended solid, since geological weathering of soil clays and their erosion, particularly during spring runoff, contribute to much of the suspended solid load in rivers. The cation exchange capacity (CEC) of some pertinent clay minerals is listed below (Ramamoorthy and Leppard 1977):

Organic Matter

Approximately 150 g of black soil was collected from the agricultural lots adjacent to the Alberta Environmental Centre, Vegreville, Alta. The soil characteristics are as follows: type = black solodized solonetz soil of A horizon; composition = sand (50%), silt (32%), and clay (18%); percent organic matter = 6.6; minerals (milligrams per kilogram) = N (27), P (8), K (128), and Na (250); pH = 7.1 and conductivity = 2 mS. The soil was extracted with 0.5 mol NaOH/L under N_2 for 18 h. The extract was then separated from the residue, and the humic and fulvic acids were isolated by the method described by Schnitzer and Skinner (1968). The ion-exchange treatment of the extract removes all the trace metal present. The freeze-dried fulvic and humic acids were added to the water column as a source of DOM. Analysis of water spiked with this organic matter showed no trace metal contamination.

Metal Analysis

Total Hg and methylmercury were determined by a differential reduction method (Environment Canada 1977; Ottawa River Project 1977) using a flameless LDC mercury monitor (model 1235, Laboratory Data Control, U.S.) equipped with a monochromatic light source (253.7 nm) and a 30-cm-long and 0.75-cm-diameter glass absorption cell with quartz windows at both ends. Reduced mercury (Hg^0) was sparged from solution with a stream of pure N_2 into the absorption cell aligned in the light path, and the absorption was read. Cd and Zn were analyzed by atomic absorption spectroscopy.

TABLE 1. Uptake of Hg by rainbow trout in presence of SS from system 1c (values in parentheses are cumulative amounts of fish and SS removed and Hg accumulated at the specified time intervals from the exposure system).

Time (h)	Fish removed (g wet wt)	Amount of Hg accumulated by fish (ng)	SS removed (g dry wt)	Amount of Hg accumulated by SS (ng)	Total amount of Hg in water (μg)	Total water L
1	0.35 (0.35)	57.9 (57.9)	2.01 (2.01)	53.2 (53.2)	2.98	4.5
3	0.42 (0.77)	58.2 (116.1)	2.02 (4.03)	146.7 (199.9)	4.04	4.3
6	0.39 (1.16)	62.5 (178.6)	2.14 (6.17)	237.2 (437.1)	3.37	4.1
12	0.34 (1.50)	75.0 (253.6)	2.11 (8.28)	172.0 (609.1)	2.91	3.9
24	0.42 (1.92)	96.8 (350.4)	2.06 (10.34)	246.1 (855.1)	2.00	3.7
48	0.35 (2.27)	71.9 (422.3)	2.06 (12.40)	159.9 (1007.1)	1.93	4.7[a]
96	0.35 (2.62)	65.6 (487.9)	2.08 (14.48)	285.8 (1292.9)	1.69	4.5
168	0.42 (3.04)	109.1 (597.0)	2.10 (16.58)	237.6 (1530.5)	0.63	4.3
288	0.69 (3.73)	160.8 (757.8)			0.39	4.1

[a]One litre of sterilized NSR water was added to replace volume lost through sampling.

Fish

Two whole fish were removed form the exposure tank at every sampling time and were pooled and homogenized in a tissue grinder with half their weight of 0.05 mol H_2SO_4/L until a smooth paste was obtained (<1 min). The wet homogenate was divided for duplicate analysis of inorganic and total Hg. Solutions were added to each aliquot (in the following order: 1 mL of 20% NaCl, 1 mL of 1% cysteine, and 10 mL of 8 mol H_2SO_4/L) and digested in a shaker water bath at 55–60°C for 1 h or more until the samples were completely dispersed. After cooling, the samples were analyzed for the released Hg–cysteine and methylmercury–cysteine complexes using different reducing agents, $SnCl_2$–NH_2OH and $SnCl_2$–Cd^{2+}, to determine the concentrations of inorganic and total Hg, respectively. The difference between these two Hg concentrations is the methylmercury content in fish.

For the analysis of Cd and Zn, the samples were drastically digested with an acid mixture of 5 mL of HNO_3 plus 2 mL of $HClO_4$ for 1 g of fish for 2 h or until white fumes of $HClO_4$ begin to appear. The digestion was continued for another 20 min, cooled to room temperature, made up to volume, and analyzed for Cd and Zn by atomic absorption spectroscopy.

Sediment

The total Hg in sediment was analyzed by a strong acid digestion with concentrated H_2SO_4 and concentrated HNO_3 with a minimal amount of concentrated HCl at 55–60°C in a shaker water bath for 2 h. After cooling, the residual organic matter was destroyed with $KMnO_4$ and $K_2S_2O_8$ solutions. The excess oxidizing solutions and the precipitated MnO_2 were neutralized with $(NH_2OH)_2 \cdot H_2SO_4$ (hydroxylamine sulphate)–NaCl solution, giving a clear extract. The extract was analyzed for total Hg content using $SnCl_2$–NH_2OH solution. Suspended solids were analyzed using the same procedure as for the sediment. For Cd and Zn analysis in sediments, the digestion method used for fish was employed with 0.5 g of sediment sample. The resins were leached overnight with 0.5 mol HNO_3/L solution at room temperature, and the leachates were analyzed for Hg, Cd, and Zn.

Detection limit and accuracy were as follows: water = 0.1 ng/mL ± 3%, fish = 0.2 ng/g ± 5%, sediment = 0.2 ng/g ± 5%. Recovery from Standard Reference Materials = 95–97% (concentration of Hg ranges from 0.24 to 1.12 μg/g). Precision was as follows:

Concentration range (ng/g)	Reproducibility (%)
0–15	93
15–37.5	93
37.5–75.0	94

Results and Discussion

The Hg burden in fish in the presence of 10 mg DOM/L was 0.14 μg/g wet wt (3-h exposure) and reached a maximum of 0.23 μg/g wet wt (24-h exposure) (Table 1). Further exposure does not increase the total Hg burden (12-d exposure, 0.23 μg/g). In the presence of 30 mg DOM/L the amount bioconcentrated in 24 h was only 0.15 μg/g wet wt whereas SS reached a steady state in just 6 h, concentrating only 0.12 μg/g dry wt, which is about half the Hg burden in fish (wet wt).

The cumulative removal of Hg by fish and SS reached 50% of their final Hg load after 48 h of exposure with 2.27 g wet wt of fish and 12.40 g dry wt of SS (Fig. 2). The average wet weight to dry weight ratio of fish was determined to be 1:0.2. The total Hg cumulatively removed on a microgram to gram dry weight basis by fish was 1.797/0.2 = 8.985 and by SS was 0.735. Rainbow trout removed up to 12 times the amount of Hg removed by SS and about 2×10^3 the amount removed by water. Lower concentrations (10 mg/L) of DOM enhances slightly the uptake of Hg by fish (Fig. 2, curves 1b and 1c). But at higher concentrations (30 mg/L) of DOM, the uptake by fish decreases markedly whereas that of SS increases (Fig. 2, curve 1d). Obviously the SS surface is competing for the fulvic (and humic) acid-bound Hg in preference to Hg^{2+}. The residence time as well as the concentration of Hg in the water column increase threefold in the presence of DOM in tanks 1c and 1d compared with tank 1b (results not shown).

Almost comparable amounts of Cd and Hg were taken up by fish after 48 h of exposure. After correcting for the background concentration, uptake of Zn was initially 30 times and reaching 40 times the concentration of Cd and Hg at the end of the experiment (Table 2). The system became slightly reducing around 24 h and eventually returned to the original redox value. The order of uptake is Zn ≫ Hg > Cd, and in the presence of 10 mg DOM/L (fulvic and humic acids) the order is Zn ≫ Cd

TABLE 2. Uptake of Hg, Cd, and Zn by fish from system 2b (values in parentheses are cumulative amounts of fish removed and HM accumulated at specified time intervals from the exposure system).

Time (h)	Fish removed (g wet wt)	Amount of HM accumulated by fish (μg)			pH	Conductivity (μS)	Redox (mV)[a]
		Hg	Cd	Zn			
6	2.49 (2.49)	1.10 (1.10)	0.85 (0.85)	55.02 (55.02)	6.90	275	419
12	2.18 (4.67)	1.73 (2.83)	1.35 (2.20)	33.61 (88.63)	7.20	275	413
24	2.50 (7.17)	1.36 (4.19)	0.90 (3.09)	37.67 (126.30)	7.19	300	378
48	1.37 (8.54)	0.75 (4.94)	0.77 (3.80)	18.49 (144.79)	7.75	210	462
168	2.09 (10.63)	0.97 (5.91)	0.91 (4.77)	40.76 (188.54)	7.76	225	449
238	2.22 (12.86)	1.09 (7.00)	1.22 (5.99)	47.47 (233.01)	7.66	225	422

[a]With reference to standard calomel electrode.

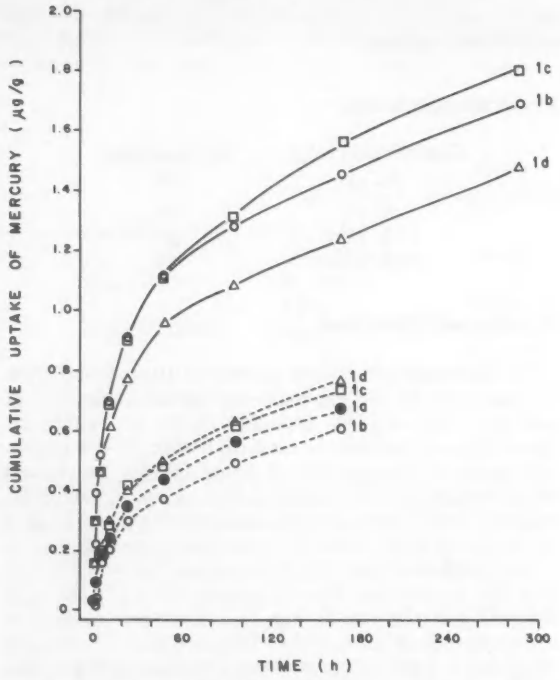

FIG. 2. Competitive uptake of Hg by fish (solid curves) and SS (broken curves) from sediment source. 1a, control tank containing unsterilized sediment spiked with 1 mg Hg/kg, SS, and 4.9 L of sterilized river water; 1b, same as 1a plus twenty 8-wk-old rainbow trout (0.2 g wet wt each); 1c, same as 1b plus 10 mg DOM/L; 1d, same as 1b plus 30 mg DOM/L.

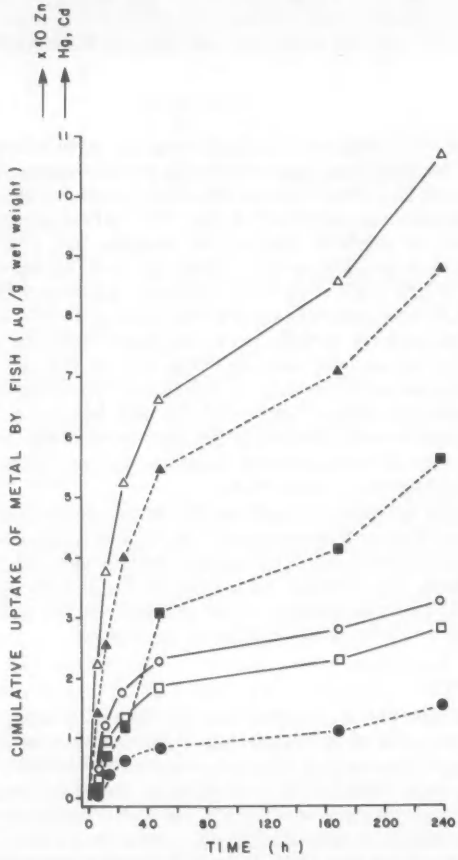

FIG. 3. Uptake of Zn, Cd, and Hg by fish and the effect of added DOM (10 mg/L). I, tank containing 4.9 L of sterilized tapwater spiked with 10 μg/L each of Zn (△), Hg (○), and Cd (□), seven dialysis sacs each containing cation exchange resin (~2 g wet wt), seven dialysis sacs each containing anion exchange resin (~2 g wet wt), two dialysis sacs each containing sediment (~2 g wet wt), and 12 rainbow trout (~1 g wet wt each) system 2b; II, same as I plus 10 mg DOM/L (system 2c) (▲, Zn; ●, Hg; ■, Cd).

≫ Hg. DOM decreased the uptake of Hg and Zn by fish but increased the uptake of Cd (Fig. 3). That Cd in water is speciated more as complexes with anionic behavior in the presence of DOM is suggested by decreased uptake by cationic resin (Table 3).

In the exposure period used in this study, fish accumulated Hg up to 0.23 μg/g wet wt (Experiment I) and 0.45 μg/g (Experiment II). This suggests possible synergism between Hg and Cd and Zn. The presence of DOM suppressed the uptake of Hg by fish. The accumulated Hg does not appear to saturate the binding site in fish, since an additional 0.55 μg Cd/g and vast quantities of Zn can be taken up. Cumulative uptake curves for Zn and Cd appear to be parallel in the presence of DOM, suggesting possible induction of metal-binding protein synthesis to deal with the metal stress (Kagi and Nordberg 1979; Olafson et al. 1979a, 1979b).

Aquatic organisms as well as mammals exhibit increased tolerance and/or resistance to one metal by previous exposure to

TABLE 3. Mass balance of Cd in exposure systems 2b and 2c (— = no data).

Time (h)	Amount of Cd (μg) Water	Fish	Cation resin	Anion resin	Sediment	Total Cd (μg)	% recovery
			Exposure system 2b				
3	39.2	—	4.87	3.51	—	47.58	95
6	29.4	4.36	7.42	5.02	—	46.02	92
12	24.5	7.25	10.52	7.52	—	49.79	100
24	19.6	5.15	16.25	8.71	—	49.71	99
48	14.7	6.28	15.08	9.15	2.19	47.4	95
96	9.8	—	16.19	7.49	—	—	—
168	9.8	5.73	16.59	9.27	2.92	44.3	89
			Exposure system 2c				
3	39.2	2.15	2.08	1.73	—	45.16	90
6	29.4	3.73	3.69	4.78	—	41.61	83
12	29.4	4.38	6.32	7.29	—	47.39	95
24	19.6	9.79	8.72	8.73	—	46.84	94
48	14.7	7.84	11.25	9.24	2.17	45.2	90
96	9.8	—	12.87	7.2	—	—	—
168	9.8	8.1	12.64	9.1	2.55	42.19	84

a different metal (Dixon and Sprague 1981; Webb 1972: Leber and Miya 1976). In mammals, exposure to Zn resulted in increased Cd resistance without affecting tolerance to Cd. Previous exposure of white suckers (*Catostomus commersoni*) to Cd, Hg, or Zn substantially increased the 96-h LC50 in Cd toxicity tests (Duncan and Klaverkamp 1983). Significantly altered tissue distribution of metal to less sensitive biochemical storage sites and induction of the synthesis of metallothionein were believed to be the causes in addition to decreased uptake and/or increased clearance of Cd. Our results do not support the last cause mentioned above.

In the laboratory, guppies (*Lebistes reticulatus*) accumulated Hg from spiked bed sediments up to 600% (1.042 μg/g) with a half-life of 38–75 d (Kudo 1976). No correlation was reported between the uptake rate and sex of fish. Younger fish accumulated Hg faster and also to higher concentrations. The Hg uptake rate ranged from 2.0 to 200 $ng \cdot g^{-1} \cdot d^{-1}$. The variation converged to 20 $ng \cdot g^{-1} \cdot d^{-1}$ with increased exposure. The percentage of methylmercury was between 44 and 70, and methylation of 0.0047% of sediment Hg per day was indicated. In our studies, an average of 73% of the total Hg in fish was methylmercury starting with a 6-h sample. Results from Experiment III showed that photochemical methylation accounted for ~5% of the total Hg. The concentrations (micrograms per litre) of total and inorganic Hg (values in parentheses) in tank I were 9.6 (8.4) at 0 h, 5.9 (5.7) at 6 h, 5.4 (5.0) at 12 h, and 4.5 (4.3) at 24 h. Hence, biological methylation of Hg must have set in during the equilibration of Hg (2 wk) with sediment.

Uptake of Zn by younger fish is reported to be greater than that by older fish (Patrick and Loutit 1978). In some cases, concentrations decrease as fish grow (Milner 1979). Recently, Rodgers and Beamish (1983) reported the relative efficiency of methylmercury uptake to be higher (1) in soft water (more than double that measured in hard water) and (2) in the presence of inorganic Hg in both soft and hard water. In hard water, addition of $ZnSO_4$ decreased slightly the uptake of CH_3Hg^+. Sublethal concentration of mercuric chloride increases mucus production in rainbow trout (Lock and Van Overbeeke 1981) and alters the structural properties of trout mucus covering the gill epithelium resulting in increased permeability to CH_3Hg^+ (Varnasi et al. 1975). Sublethal concentrations of Zn have also been reported to increase the mucus production in trout (Sellers et al. 1975) but decrease uptake of CH_3Hg^+ in the presence of $ZnSO_4$ in hard water (Rodgers and Beamish 1983). This could result from Ca^{2+}-induced changes in gill permeability and charge resulting in decreased permeability to CH_3Hg^+ and other cations in hard water (McWilliams and Potts 1978). We observed an increase in Hg uptake due to the fact that the water was soft, and also the spiked Hg was inorganic.

Accumulation of greater quantities of Zn in our studies (40 times over Cd and Hg) by fish confirms the intermediate property of Zn unlike Cd and Hg to bind with both oxygen and sulphur ligands of biomolecules (Ahrland 1966; Pearson 1968a, 1968b). The lower affinity of methylmercury for metallothionein than either Cd^{2+} or Hg^{2+} (the binding order is $Hg^{2+} > Cd^{2+} \gg CH_3Hg^+$) suggests that Zn-induced metallothionein can hold CH_3Hg^+ only weakly (Chen et al. 1973). This might lead to possible redistribution or increased clearance of methylmercury from fish.

We conclude that (1) organic matter decreased the uptake of Hg and Zn by fish but enhanced the uptake of Cd; (2) equilibration with one HM ion did not limit the fish in accumulating another HM ion; possibly there exists different or multiple binding sites in fish for different HM ions; (3) mass balancing for the HM ions studied revealed excellent recoveries for Cd and Zn but not Hg. Losses up to 50% towards the end of experiment could have been due to volatilization of Hg as Hg^0 (Ramamoorthy et al. 1977); (4) some (~5%) photochemical methylation of Hg could be detected; (5) calculated approximate partition coefficients (fish/substrate) were as follows:

HM ion	SS	Sediment	Cation resin	Anion resin	Water
Hg	12	3.0	2.5×10^4	2.5×10^4	2×10^3
Cd	—	2.8	2	3.6	550[a]
Zn	—	1.5	0.6	1.0	99

[a]Water was free from spiked organic matter.

These values show that compartments in the ecosystem are critical in the bioavailability of a given HM ion. For Hg, sediment and SS are the only compartments determining its flux, whereas for Cd and Zn, all the compartments studied here are critical. The impact of the different compartments of the ecosystem on the HM ion distribution followed the order Hg < Cd < Zn.

The experimental design developed in this study has a great potential for use as a model ecosystem to study the competitive interactions on the uptake of trace pollutants by biota. Fate processes such as volatilization can be estimated by mass balancing the test chemical. Once the "steady state" is reached (24 h as in this study), the additional points serve to validate the reproducibility of the data. Methodology is simple and yet yields a vast amount of data depending on the number of compartments included. By proper manipulation, a testable model could be developed for the uptake of multiple trace pollutants. The results are relevant to natural conditions, since the data could serve as predictors of residence time and the extent of fate processes in the presence of competing accumulators.

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Biological Availability of Soluble Reactive Phosphorus in Anoxic and Oxic Freshwaters[1]

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Nürnberg, G. K., and R. H. Peters. 1984. Biological availability of soluble reactive phosphorus in anoxic and oxic freshwaters. Can. J. Fish. Aquat. Sci. 41: 757–765.

The availability of hypolimnetic phosphorus was assessed by a short-term bioassay, based on ^{32}P uptake kinetics. In the anoxic hypolimnia of eight lakes and in anoxic model systems (core tubes), at least 80% of soluble reactive phosphorus is biologically available. However, biologically available phosphorus is only a small fraction of soluble reactive phosphorus in iron-rich, previously anoxic waters after aeration and in lake water from the anoxic–oxic interface. This result is supported by published data for oxic waters from epilimnia of lakes, rivers, sewage effluent, and precipitation.

Nous avons évalué l'accessibilité du phosphore hypolimnétique à l'aide d'une analyse biologique à court terme fondée sur la cinétique de captation de ^{32}P. Dans l'épilimnion anoxique de huit lacs et dans des systèmes modèles (tubes de carottes), au moins 80 % du phosphore réactif soluble (SRP) est biologiquement accessible (BAP). Cependant, BAP n'est qu'une petite fraction de SRP dans des eaux riches en fer, auparavant anoxiques, après aération et dans de l'eau de lac provenant de l'interface anoxique–oxique. Des données publiées sur des eaux oxiques provenant d'épilimnions de lacs, ainsi que de rivières, d'effluents d'eaux usées et de précipitations appuient ces résultats.

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Soluble reactive phosphorus (SRP) consists not only of orthophosphate (PO_4) but also of some acid-labile phosphorus compounds (Olsen 1967; Harwood et al. 1969). Several workers have found that SRP analysis overestimates PO_4 concentrations (Kuenzler and Ketchum 1962; Rigler 1966, 1968; Peters 1977, 1978, 1979; Tarapchak and Rubitschun 1981) although in some cases, SRP and PO_4 were identical (White and Payne 1980). Since phosphorus compounds other than PO_4 might become available during a bioassay (Paerl and Downes 1978), SRP need not overestimate biologically available phosphorus (BAP), even if SRP overestimates PO_4 (Stainton 1980). Some reports show that SRP and BAP are indeed identical (Walton and Lee 1972; Chamberlain and Shapiro 1973).

In this paper, we investigate the equivalence of SRP and BAP. Our experimental work is largely confined to anoxic lake water from hypolimnia and cores containing phosphorus released from the sediment. The short-term radiological bioassays we used to measure BAP are specific for PO_4 and kinetically similar substances. To cover a larger range of phosphorus concentrations, water bodies, and techniques, we also compare published concentrations of SRP with BAP or PO_4.

Materials and Methods

Sample Water

To estimate the bioavailability of phosphorus in anoxic hypolimnia, water was collected during summer stratification from two lakes in Quebec (weekly, 1981; Lake Magog and Fitch Bay of Lake Memphremagog) and six lakes in Ontario (weekly to monthly, 1982: Little Clear, Chub, Blue Chalk, Williams Bay of Jack Lake, Glen, and St. George). Lakes and sampling techniques are described in Nürnberg (1984). We also examined SRP and BAP released from anoxic lake sediments. Cores containing undisturbed sediment and hypolimnetic water from Fitch Bay were incubated under anoxic conditions in the dark for 2–15 d. On several occasions, surface water from nitrogen-limited Lake Magog was also analyzed.

SRP Analysis

SRP (like BAP) was analyzed within 5 h after sampling. In anoxic waters, ferrous iron and hydrogen sulfide interfere with SRP analysis (Nürnberg 1984). Therefore, samples that were rich in iron were kept and filtered under anoxic conditions to avoid phosphorus adsorption to ferric iron, and subsequent underestimation of SRP; samples that were rich in hydrogen sulfide were bubbled with air to drive off H_2S (Nürnberg 1984). Filtrates were analyzed for SRP by the molybdenum blue method following Stainton et al. (1977). The precision was less than 1% of the mean (standard error of four repeated analyses at concentrations >10 μg P/L); the detection limit was 0.75 μg P/L. To determine if aeration affects the availability of SRP, about 100 mL of hypolimnetic water was aerated with an aquarium pump for 0.5–5 h before SRP and BAP analysis.

BAP Analysis

Our analysis of BAP in the sample water (test water) is based on the change in the amount of $[^{32}P]PO_4$ uptake by plankton in

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phosphorus-limited lake water (assay water) in response to additions of $[^{31}P]PO_4$. This bioassay is essentially that described by Peters (1977, 1978, 1979), although it avoids filtration of the test water and the dilution of assay water with the filtrates. The assay is also similar to that described by Dillon and Reid (1981), except that the proportion of test water added to the assay water is smaller and the standard curves are based on the proportion of tracer taken up in a specific interval rather than on the rate constant of uptake. It is fundamentally different from Rigler's (1966) assay because it compares uptake after addition of the test water with uptake after the addition of a PO_4 standard; the estimation of BAP concentrations are therefore based on interpolations rather than extrapolations. In this assay, the fraction of $[^{32}P]PO_4$ in solution at a given time correlates positively with the amount of $[^{31}P]PO_4$. Carrier-free $[^{32}P]PO_4$ in 0.1 mol HCl/L was obtained from the New England Nuclear Corporation and diluted to 0.001 mol/L before experiments were begun. Standard curves were generated by adding known amounts of $[^{31}P]PO_4$ and $[^{32}P]PO_4$ to assay water. Simultaneous runs using additions of $[^{32}P]PO_4$ plus test water are then compared with the standard curves and the PO_4 concentration in the test water determined by interpolation. A typical result is shown in Fig. 1. This assay analyses only PO_4 and phosphorus compounds with similar uptake kinetics; it indicates the minimum phosphorus available (Peters 1981) and does not quantitatively detect phosphorus compounds with slower availability whose kinetics deviate from the PO_4 standard (Lean 1973; Paerl and Downes 1978).

Anoxic "test water" was added unfiltered to avoid filtration artifacts upon aeration; epilimnetic test water was filtered (Millipore, 0.45 μm) before addition. The standard error of the BAP analysis was at most 10% of the mean; this is based on the average deviation of 10 replicate analyses at concentrations above 10 μg/L.

Orthophosphate standards were made from a stock solution of dehydrated K_2HPO_4, dissolved in double-distilled water, and diluted to approximate the SRP concentration of the test water. Standard curves were produced by addition of several volumes (0, 1, 2 mL) of this solution to 100 mL of each assay water. In all cases but one, the assay water consisted of water from the epilimnion of the same lake from which the hypolimnetic water was taken. In nitrogen-limited Lake Magog, $[^{32}P]PO_4$ uptake by microorganisms was too slow. Therefore, epilimnetic water from another lake, usually Fitch Bay, was used instead. The volume of test water was diluted 10- to 1000-fold with the assay water depending on the hypolimnetic SRP concentration. This dilution depressed the iron concentration in iron-containing hypolimnetic water below the threshold (0.20 mg Fe/L) for significant adsorption of phosphorus onto Fe particles (>0.45 μm) within the period of the bioassay (Nürnberg 1984). To detect possible dilution effects, each determination involved at least two different volumes of test water, although no significant deviations were ever detected. Because uptake rates are dramatically effected by temperature (G. Nürnberg, in preparation) and possibly by light and time since collection, standards, blanks, and two test water samples were processed simultaneously. Stirring or shaking during the assay did not affect uptake and was not carried out routinely.

At zero time, 100 μL of $[^{32}P]PO_4$ solution was added to the standard and test solutions. At each subsampling time, 6-mL subsamples were withdrawn and filtered (<35 kPa) simultaneously through 0.45-μm Millipore filters at three to six

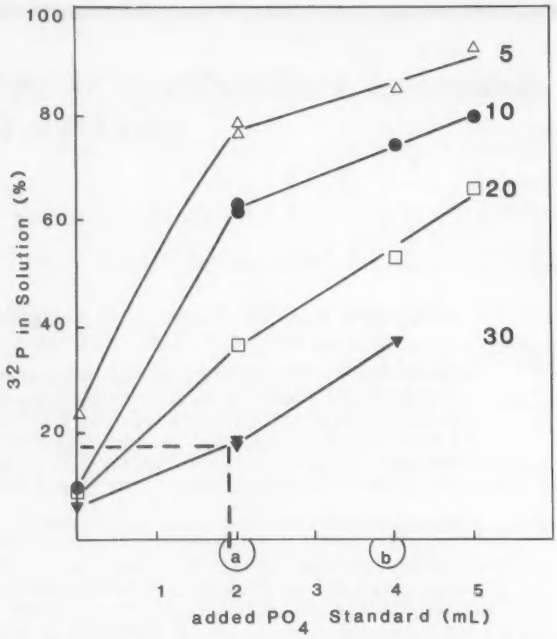

FIG. 1. Typical bioassay with Jack Lake epilimnetic water as assay water (5 m, Aug. 26, 1982) calibrated with additions of 0, 2, 4, and 5 mL of 75 μg PO_4/L standard solution. Subsampling times were 5, 10, 20, and 30 min. The additions of 2 (a) and 4 (b) mL of anoxic hypolimnetic water (20 m) sampled at the same location are comparable with additions of 1.95 and 3.9 mL of the standard. This indicates that the test water concentration is 73 μgPO_4/L. Broken lines indicate how to arrive from the uptake value at 30 min (a) at the corresponding PO_4 concentration.

different times (usually 5, 10, 20, 30 min) within 1 h. The filter holders had been checked for filtering efficiency and reproducibility, and only those that had reproducible retention of 10 or 5% (for different manifolds) were selected. The sample was filtered directly into plastic minivials and the ^{32}P radioactivity measured by Cerenkov counting on the tritium channel of a liquid scintillation counter to 1% counting error. The counts of the filtrate, corrected for filter adsorption of some soluble tracer, were expressed as a percentage of the mean of two unfiltered 6-mL samples, taken before and after the assay.

To reduce the possibility of contamination, the assay vessels (250-mL Erlenmeyer flasks) were filled with an approximately 0.5% solution of a basic solvent (Count Off, New England Nuclear) during storage. Before each bioassay, the vessels were rinsed three times with distilled water and once with the assay water. Assay water (100–200 mL) was placed in the vessels for at least 15 min (the late F. H. Rigler, McGill University, pers. comm.) so that available phosphorus could be reduced still further by the plankton. This water was discarded and exactly 100 mL of assay water was added and allowed to remain in the reaction vessel for at least 0.5 h before addition of tracer and carrier. Flasks and pipettes for subsampling were kept covered during the whole assay.

Hypolimnetic water might contain substances besides PO_4 that affect $[^{32}P]PO_4$ uptake kinetics. This was evaluated in the

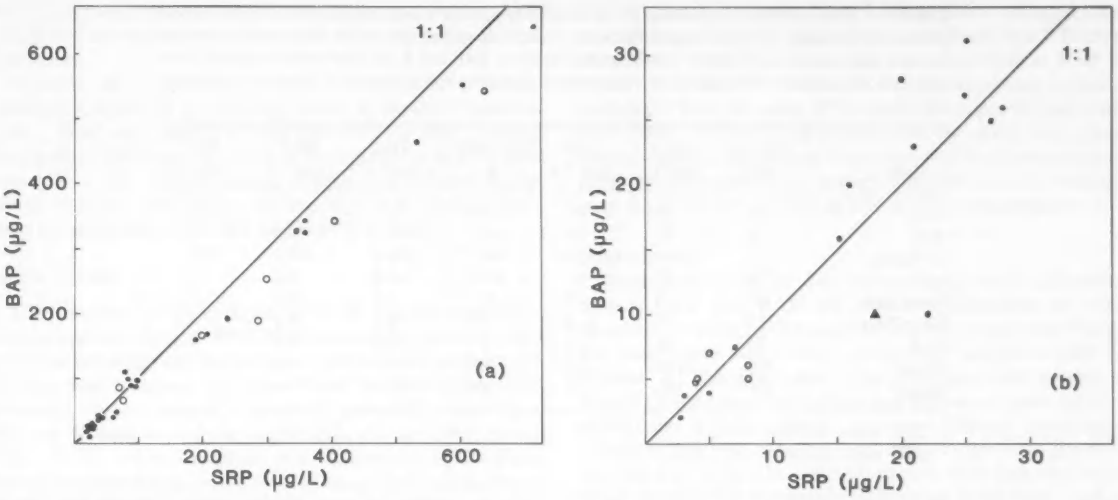

FIG. 2. Correlation of SRP with BAP. The line of perfect agreement (1:1) is also shown. (a) Water from anoxic hypolimnia of eight lakes (●) and anoxic core tubes (○); (b) water from the same anoxic lakes with SRP concentrations less than 30 µg/L (●), oxic epilimnia (○), and surface water during fall turnover (▲) of Lake Magog.

following ways:

1) The uptake kinetics after addition of the test water were compared with uptake kinetics of the standard water. Deviation from the shape of the standard curves indicates interference and no BAP concentration was calculated. These comparisons suggest that two points from Fitch Bay (n = 9) and one from Little Clear Lake (n = 8) were not reliable; these data were discarded. Since both lakes contain high iron concentrations, it is possible that the different kinetics reflect a phosphorus fraction that is available at a slower rate than PO_4 (Paerl and Downes 1978).

2) Another test for interfering and poisonous substances was achieved by additions of internal standards. A known concentration of [^{31}P]PO_4 was added with the test water for each lake at least once, but a deviation from the results without internal standards was detected on only one occasion: the hypolimnetic water of Glen Lake, where large amounts of hydrogen sulfide slowed uptake of tracer and thus produced an overestimate of BAP concentration. This problem was subsequently prevented by aerating the samples for at least 30 min before addition to the assay water.

3) Bacteria are primarily responsible for initial ^{32}P uptake (Hayes and Phillips 1958; Lean and White 1983). Therefore, on two occasions, substances that are likely to support bacterial growth such as carbohydrates, nitrate, iron, and calcium were added to the assay water at concentrations similar to those in hypolimnetic water; no deviation was found.

4) We determined whether differences in phosphorus limitation of the assay water or differences in the plankton community or water chemistry have any effect on the bioassay. This was tested on several occasions by analyzing BAP in the same hypolimnetic water in separate bioassays with water from the epilimnia of two different lakes. Although the assay waters differed in the amount of ^{32}P uptake by 60% within the first hour, no significant difference in the BAP concentration was found. This means that incubation and growing of cultures under standardized conditions can be circumvented by using phosphorus-limited epilimnetic water as assay water.

Results and Discussion

Short-Term ^{32}P-Bioassays

SRP concentrations for all anoxic lake samples (range 2–531 µg SRP/L) are similar to (Wilcoxon matched pairs signed ranks test: $P > 0.122$, $n = 33$) and highly correlated with (r^2 = 0.994, n = 33, Fig. 2a) BAP concentrations. The linear regression of BAP on SRP is BAP = 2.501 + 0.923SRP, (SE_{slope} = 0.0131). This suggests that BAP exceeds SRP at concentrations below 30 µg SRP/L. Since BAP concentrations did not consistently exceed SRP at lower concentrations, linear regression over the whole range does not adequately describe the data. Therefore, the data set was divided into two subsets, consisting of values above and below 30 µg/L SRP, and reanalyzed. Again, the Wilcoxon test revealed no significant difference for SRP concentrations <30 µg/L (n = 13, $P >$ 0.422), but the difference was significant for higher SRP concentrations (n = 20, $P > 0.032$). The regression line for the low concentration subset coincides with the 1:1 line (Fig. 2b, BAP = 0.665 + 0.993SRP, SE_{slope} = 0.1558, r^2 = 0.787, n = 13). For concentrations above 30 µg SRP/L, the regression equation (BAP = 3.441 + 0.921SRP, SE_{slope} = 0.01977, r^2 = 0.992, n = 20) indicates that SRP overestimates BAP concentration up to an average of 8% for the higher concentrations.

The tendency for SRP to overestimate BAP at high concentrations was also found in water from core tubes containing sediment and anoxic water (Fig. 2a). The highly significant regression for seven cores (r^2 = 0.98, BAP = 0.305 + 0.841SRP, n = 7, range 71–636 µg/L SRP) shows that 84% of SRP, on the average, is available. The discrepancy between SRP and BAP at high SRP concentrations is unexplained, but it may represent a systematic experimental error. At such high

TABLE 1. SRP and BAP concentrations in artificially aerated water samples from anoxic hypolimnia of seven lakes. The SRP concentration before aeration was similar to that after aeration, except for Jack Lake and Lake Magog. The difference between SRP and BAP after aeration depends on total iron concentration (TFe) and the concentrations of soluble iron before (SFe^{2+}) and after aeration (SF^{3+}).

Lake	Aeration (min)	SRP (μg/L)	BAP (μg/L)	BAP/SRP %	TFe (mg/L)	SFe^{2+} (mg/L)	SFe^{3+} (mg/L)
Glen	35	39	40	102	<0.1	<0.1	<0.1
Glen	35	92	90	98	<0.1	<0.1	<0.1
St. George	35	343	329	96	<0.1	<0.1	<0.1
Jack[a]	150	59	60	102	0.4	0.2	0.2
Blue Chalk	92	16	2	13	3.4	3.0	3.0
Little Clear	35	25	1–2	6	5.2	4.9	4.9
Chub	35	31	3	9	5.5	4.9	4.8
Magog[b]	300	193	125	65	4.0	4.0	0.7
Magog[b]	300	192	70	36	3.9	—	—

[a]SRP before aeration: 88 μg/L.
[b]SRP before aeration: 672 μg/L.

concentrations, only 100 μL of test water was pipetted into the assay water and enough oxygen could have diffused into such a small volume to produce significant iron–phosphorus precipitation. This is suggested because the overestimate was pronounced in waters with high iron concentrations. In any case, the results show that at least 80% of SRP is biologically available.

In some anoxic hypolimnetic waters, oxidation of ferrous iron could interfere with SRP and BAP analyses, since ferric iron·hydroxides can adsorb PO_4 (Stumm and Morgan 1970) rendering it insoluble and less available. Experiments were designed to determine the effect of aeration of the test water on the close agreement between SRP and BAP. In hardwater lakes (Glen, St. George), SRP and BAP concentrations remained unchanged. In the other lakes, aeration decreased BAP (Little Clear, Chub, Blue Chalk) or both SRP and BAP concentrations (Magog, Jack). Where BAP decreased but SRP stayed the same, subsequent SRP analysis drastically overestimated BAP concentration. Decrease of the BAP concentration depended on the total iron concentration in the hypolimnetic water (Table 1). In softwater lakes that were rich in organic acids (Little Clear, Chub, Blue Chalk), SRP and soluble iron remained unchanged, although BAP decreased and all the iron was converted to the ferric state (Nürnberg 1984). This suggests that, in these lakes, the iron–phosphorus particles were initially smaller than 0.45 μm. In Lake Magog, an uncolored lake, the iron–phosphorus particles were larger than 0.45 μm. As a result, SRP decreased after aeration and SRP overestimated BAP to a lesser extent (Table 1).

Although this discrepancy between SRP and BAP was induced artificially, iron-rich hypolimnetic water may be aerated naturally at the anoxic–oxic boundary during the stratified period and at turnover. In Lake Magog, bioassays of hypolimnetic water from the oxic–anoxic boundary and of surface water during destratification showed that less than 70% of SRP was BAP (Table 2). It appears that BAP concentration is lower than SRP in these cases because of formation of colloids and particles containing iron and phosphorus. Such phosphorus might become available in the long run but this was not investigated.

Artificial formation of colloidal iron–phosphorus compounds

TABLE 2. SRP and BAP concentrations in naturally aerated, iron-rich water of Lake Magog (1981). The data were collected at the oxic–anoxic interface in the hypolimnion and in the epilimnion at fall turnover.

Date	Depth (m)	O_2 (mg/L)	SRP (μg/L)	BAP (μg/L)	BAP/SRP %	Fe (mg/L)
June 9	16	6	13	4	33	0.29
June 25	16	6	89	50	56	0.71
July 3	16	2	115	41	35	0.79
July 3	12	4	25	9	37	0.30
July 8	16	1	140	73	52	1.06
July 8	12	2	14	9	69	0.27
Fall turnover		10	18	10[a]	53	0.22
		10	18	11	61	0.22

[a]Filtered before analysis.

was also observed by Koenings and Hooper (1976), who found that aeration of anoxic bog water increased the colloidal phosphorus fraction from 0 to 33% (range of SRP: 266–605 μg/L). White and Payne (1980) found that "reactive high molecular weight phosphorus" (perhaps a colloidal iron–phosphorus component) accounted for up to 76% of the SRP concentration in three of five eutrophic anoxic hypolimnia of New Zealand lakes. This was not observed in the presumably oxic hypolimnia of mesotrophic or oligotrophic lakes and occurred only once in seven oxic epilimnia (at turnover) (E. White, Department of Scientific and Industrial Research, Taupo, New Zealand, pers. comm.). This may indicate the artificial formation of phosphorus–iron colloids upon aeration of anoxic hypolimnetic water during analysis, since the two lakes with the highest deviation of PO_4 from SRP have substantial iron concentrations even in the epilimnia (1.6 and 0.4 mgFe/L) (E. White, pers. comm.). On the other hand, Francko and Heath (1982) found that SRP increases with the reduction of iron by UV radiation and that phosphorus in high molecular weight fractions containing ferric iron is not analyzed

as SRP, although it passes a 0.45-μm filter. In such waters, SRP should not overestimate BAP in spite of the presence of iron hydroxides.

These results suggest that, in anoxic water, SRP is almost completely available in the short term. In aerated, iron-rich waters, BAP may represent less than 10% of SRP. During stratification, SRP can be completely available in oxic surface waters: five tests of water from the epilimnion of Lake Magog during summer stratification in the range of 4–8 μg/L show good agreement between SRP and BAP (Fig. 2b).

Literature Data

The literature survey includes 603 BAP and SRP data pairs from phosphorus-limited lakes, nitrogen-limited lakes, sewage treatment effluents, atmospheric precipitation, and streams. All the data from bioassays are called "BAP" in this article, since bioassays may not identify chemically pure PO_4, even when they are thought to analyze largely PO_4 (Rigler 1966; Peters 1977, 1978, 1979; Tarapchak and Rubitschun 1981). Four classes of data were excluded from the analysis: (1) some data of White and Payne (1980), which may be subject to iron hydroxide interference as described above; (2) BAP values based on Rigler's (1966) bioassay have been excluded because they are based on statistically questionable extrapolations. This deletion has no significant effect on the overall analysis, since the number of data is small; (3) SRP analyzed with the extraction technique (Chamberlain and Shapiro 1969); (4) those data with reported arsenic interference. Regression analysis and mean differences (SRP – BAP) were used as a measure of the overall (positive or negative) deviation of BAP from SRP. Subsets of these data for low and high SRP concentrations, for different laboratories, BAP methods, and types of water, were investigated to determine whether these characteristics are important.

Overall correlations

The overall correlation of the SRP and BAP values is highly significant ($r = 0.97$). The mean difference (SRP − BAP) is 2.18 μg/L, which is significant by the Wilcoxon matched pairs signed ranks test ($P < 0.0001$). A parametric test is not appropriate because the data are very widespread and not normally distributed. The linear regression equation is BAP = 0.73 + 0.91SRP ($SE_{slope} = 0.008$, $r^2 = 0.96$, $n = 601$, range 0–700 μg/L SRP). Two significantly influential outliers were excluded (Cook's (1977) criterion). The intercept is not significantly different from zero but the slope differs significantly from unity. The data are presented in a log–log plot in Fig. 3a (53 data values of SRP or BAP were zero and had to be excluded from the logarithmic plot). We conclude that there is a close agreement of SRP and BAP values and the difference, although significant, is only small.

It has been suggested that the SRP concentration may influence the similarity of SRP and BAP (Chamberlain and Shapiro 1973). To investigate this, the data set was divided into two subgroups analogous to those formed ealier for the anoxic lake data set. The mean difference (SRP − BAP) for SRP concentrations <30 μg/L (mean SRP = 6.2 μg/L) is 0.03 μg/L which, although small, is significant by a Wilcoxon test ($P < 0.002$). The linear regression equation is BAP = −0.15 + 1.04SRP ($r^2 = 0.52$, $n = 490$) and the slope and intercept are not significantly different from unity and zero, respectively (Fig. 3b). For SRP values >30 μg/L (mean SRP = 165 μg/L), the mean difference is 11.5, which is also significant by a Wilcoxon test ($P < 0.001$). The linear regression equation is BAP = 13.04 + 0.78SRP ($r^2 = 0.80$, $n = 112$; one significant outlier is excluded). Both the mean differences and slopes indicate that, when SRP > 30 μg/L, the absolute amount of the overestimation increases. A similar result was obtained for the anoxic lakes presented above; hence, it appears that SRP tends to overestimate BAP slightly (<20%) at higher SRP concentrations.

Data sources

Range and mean of SRP values and mean differences between SRP and BAP are presented separately for each laboratory (Table 3). This analysis allows comparison of large and small data sets when a regression analysis would be misleading. The mean percentage difference with respect to mean SRP concentration (percentage difference: mean [SRP − BAP]/mean [SRP]) corrects for the different phosphorus concentrations and indicates any bias (negative or positive) between SRP and BAP. Different sources show quite heterogeneous results. The percentage differences range from −23 to 87%, indicating that SRP overestimates BAP in some cases and underestimates it in others; also, BAP is not always strongly correlated with SRP. To determine whether these differences are due to special water types (e.g. water rich in fresh iron hydroxides) or the result of the applied methods, the data were grouped by BAP method and water types.

BAP methods

The variability in estimating BAP from SRP is quite large among different methods for BAP analysis (Table 4). BAP estimated from Rigler's (1966) tracer biassay and from ^{32}P biassays of filtered water were considerably less than SRP concentrations, while BAP estimates based on acetylene reduction were, on average, greater than SRP measurements. Sephadex fractionation and ^{32}P bioassays using unfiltered water usually gave concentrations of BAP that were close to those of SRP. Part of the discrepency between SRP and BAP measured with either ^{32}P bioassay may be associated with low SRP levels. At such levels, both SRP and BAP estimates are more variable (Fig. 3), probably reflecting both greater analytical error and the more pronounced effects of contamination. Low SRP levels are not always poor estimators of BAP concentrations, since Sephadex fractionation shows only small differences between SRP and BAP (percentage difference = 3%) at low concentrations (mean [SRP] = 1.9, range 0.3–5.0 μg/L, $n = 11$) and, overall, the mean percentage difference at SRP levels below 30 μg/L was only 1%. Further work may be required to determine if the large percentage differences reported using these ^{32}P bioassays represent pecularities of given lakes or methodological shortcomings.

To circumvent the problem of introducing unknown plankton into the bioassay vessel, many researchers filtered the water to be tested before the bioassay. Filtration before analysis does not affect BAP estimates and might be unnecessary (Table 4). However, the percentage difference of ^{32}P uptake bioassay with filtered water was greater than with unfiltered water.

SRP methods

All the SRP methods used in these studies were based on the molybdenum blue method with stannous chloride (Harvey 1948) or ascorbic acid (Strickland and Parsons 1972) as

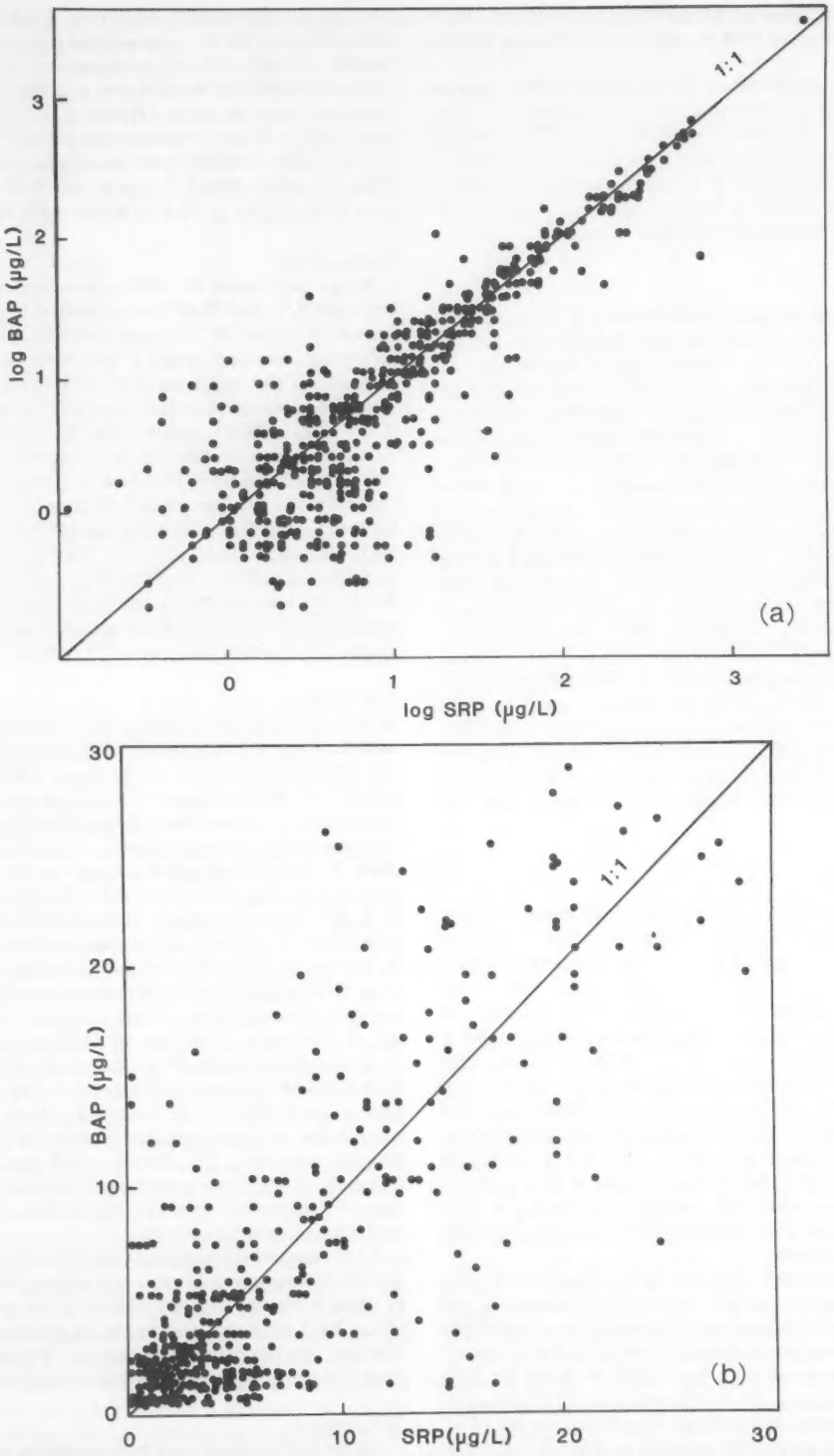

FIG. 3. Correlation of SRP with BAP with data from Table 3. The line of perfect agreement (1:1) is also shown. (a) log–log plot: log BAP = −0.11 + 0.97SRP, n = 550, r^2 = 0.70; (b) linear plot for SRP concentrations less than 30 µg/L: BAP = −0.15 + 1.04 SRP, n = 490, r^2 = 0.52.

TABLE 3. Comparison between concentrations (μg/L) of SRP and BAP reported by different authors (n = number of data pairs; r = correlation coefficient for SRP and BAP; mean (x_2) and standard error (SE) of SRP − BAP is expressed as the percentage of mean SRP ($x_2/x_1 \times 100$); f = filtered; uf = unfiltered; AGP = algal growth potential; NS, not significant; NA, not available).[a]

Source	Range [SRP]	n	r	Mean [SRP] x_1	SRP − BAP x_2	SE	% x_2/x_1	Assay type	Assay organism	Period
1	2–636	44	0.99	144.3	12.9	3.95	9	^{32}P uptake, uf	Lake plankton	1 h
2	0.6–54	181	0.73	7.2	3.2	0.51	44	^{32}P uptake, f	Lake plankton	1 h
3	1–181	25	0.99	45.4	0.2	0.85	0	AGP	*Selenastrum*	21 d
4	1–30	5	0.98	13.5	1.9	0.92	14	SRP uptake	*Microcystis*	2 h
5	0–592	16	0.99	46.1	1.0	0.75	2	Sephadex fractionation	*Chlorella*	96 h
6	3–3000	6	0.99	542.3	−123.3	115.52	−23	Acetylene reduction	*Anabaena*	1–2 h
7	0–90	49	0.87	12.1	−2.6	1.56	−21	^{32}P uptake, uf	Lake plankton	15 min
8	0–340	209	0.96	23.5	0.5	1.02	2	^{32}P uptake, uf	Lake plankton	15 min
9	0–500	22	0.95	1.2	−0.2	0.08	17	AGP	*Scenedesmus*	14–28 d
10	414–549	2	—	481.5	0	0	0	Sephadex fractionation	None	—
11	0–1000	44	0.46	54.0	19.2	15.66	36	AGP	*Selenastrum*	14 d
12	0.3–5	9	NS	1.78	1.3	0.49	74	Rigler bioassay	Lake plankton	1–2 h
13	1–8	21	NA	3.6	3.1	NA	87	Rigler bioassay	Lake plankton	1–2 h
14	0–11	13	NS	2.7	1.6	0.76	58	Rigler bioassay	Lake plankton	1–2 h

[a]Sources: 1, this study; 2, Peters (1977, 1978, 1979); 3, Walton (1971). (Algae counts given by the author were converted into BAP concentrations for Lake Mendota samples. Since Walton (1971) considers data for Lake Wingra unreliable these are not included.); 4, Chamberlain and Shapiro (1969) (SRP analyzed with Harvey's method for low arsenate concentrations only); 5, White and Payne (1980). (Three values for anoxic hypolimnia were excluded because of possible aeration artifacts (iron adsorption, see text). The phosphate extracted here was proven to be completely available to *Chlorella* (Paerl and Downes 1978).); 6, Stewart and Alexander (1971); 7, Dillon and Reid (1981); 8, P. J. Dillon, Ontario Ministry of the Environment, pers. comm.; 9, DePinto et al. (1980); 10, Murphy et al. (1983); 11, Twinch and Breen (1982); 12, Rigler (1966); 13, Tarapchak and Rubitschun (1981); 14, Peters (1979).

TABLE 4. SRP and BAP mean differences sorted by method of BAP analysis (symbols as in Table 3; data from source 13 are not included, since only ranges and means are available).

Method	n	r	Mean [SRP] x_1	SRP − BAP x_2	SE	%	Source
^{32}P, uf	302	0.99	39.2	1.80	0.98	5	1, 7, 8
^{32}P, f	181	0.73	7.2	3.2	0.51	44	2
Sephadex fractionation	18	1.00	94.5	0.87	0.67	1	5, 10
AGP	91	0.53	38.9	9.32	7.60	24	3, 9, 11
Rigler assay	22	NS	2.3	1.46	0.49	64	12, (13), 14
Acetylene reduction	6	0.99	542.2	−123.3	115.52	−23	6
Unfiltered	320	0.99	42.3	1.75	0.93	4	1, 5, 7, 8, 10
Filtered	283	0.97	28.9	2.45	3.51	8	2, 3, 4, 6, 9, 11

reductant. Although $SnCl_2$ has been reported to yield slightly higher values than the ascorbic acid method, they differ by not more than 20% (Stainton 1980; Tarapchak et al. 1982b). Other factors can lead to deviation of apparent SRP concentrations: the presence of arsenate (Chamberlain and Shapiro 1969) and calcium (Burnison 1983), high-volume and high-pressure filtration (Tarapchak et al. 1982a), and extended exposure to acid and unclean conditions (Rigler 1964). These effects on the published SRP data reported here cannot be evaluated, but they probably contribute to the scatter in the data, particularly at low SRP concentrations.

Water types

Close agreement between BAP and SRP was found in most waters (Table 5), although SRP may overestimate BAP in groundwaters (Peters 1979) and in artificially enriched limnocorals (Twinch and Breen 1982). These data are drawn from the work of single laboratories and may confound other differences in methods or sampling sites with the effect of water type. It is also possible that aeration during sampling of groundwater induced phosphorus precipitation with iron and so reduced BAP levels, since work with cores in the same area (G. Nürnberg, unpubl. data) and general surveys (White et al. 1963) indicate that anoxic groundwater is often rich in ferrous iron. More evidence is required to evaluate the ability of SRP to measure BAP in artificially enriched enclosures. Underestimation of BAP by SRP in sewage effluents seems frequent, although the large standard error of the difference suggests that it may not be significant.

Although no experimental verification was done, the variation in predicting BAP from SRP in this data set seems to originate from the different methods used by different authors as well as from different natural water types. SRP analysis does not result in pronounced overestimate of BAP, especially at

TABLE 5. SRP and BAP mean differences sorted by the type of water (symbols as in Table 3; references 12–14 are not included).

Type	n	r	Mean [SRP] x_1	SRP − BAP x_2	SE	%	Source
Hypolimnion	51	0.99	144.4	11.1	3.47	8	1, 3, 5
Epilimnion	98	0.99	12.1	0.4	0.51	3	1, 2, 3, 4, 5, 6
Streams	283	0.96	18.0	1.6	0.73	9	1, 2, 6, 8
Sewage	24	0.99	126.6	−29.1	29.17	−23	4, 6, 9
Sediment, soil	4	0.99	284.5	−0.3	1.03	0	3, 10
Blue-green extract	2	—	33.0	5.5	—	17	3
Precipitation	100	0.95	15.6	0.1	1.37	1	2, 7, 8
Groundwater	6	NS	3.7	2.1	0.97	57	2
Limnocorals	35	0.64	64.3	26.1	19.55	41	11

concentrations less than 30 μg/L SRP in lakes, streams, precipitation, and soil and sediment extractions. The study suggests that most available phosphorus is analyzed as SRP during the period of the BAP assay. Therefore, SRP analysis can be used to estimate bioavailable phosphorus over a range of three orders of magnitude in phosphorus concentration for oxic and anoxic freshwater of all types and presents a faster tool than bioassays. However, short-term BAP is substantially overestimated by SRP analysis in the presence of freshly formed iron hydroxides. In these cases the short-term bioassay described here can be applied.

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Forms of Aqueous Aluminum in Acidified Catchments of Central Ontario: A Methodological Analysis

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Separating the different forms of aqueous aluminum is essential for determining their relative toxicity in acidified streams and lakes. A short extraction with oxine at pH 8.3 separates inorganic monomeric and organic aluminum from the polymeric and amorphous forms. The organic fraction can be eliminated by equilibrium dialysis prior to extraction. In many cases, the inorganic fraction can also be estimated from free and total fluoride measurements. The two methods agree well in artificial solutions (R^2 = 0.997) and reasonably well in natural waters (R^2 = 0.82), although the computed estimates tend to be slightly lower than the directly measured values. During spring snowmelt in the Muskoka–Haliburton region of central Ontario, very little polymeric and amorphous aluminum was found, and most of the inorganic monomeric fraction was in the fluoride complexes. I present an empirical model that predicts inorganic monomeric aluminum in these waters (R^2 = 0.90) using total organic carbon and the aluminum measured by a short extraction without dialysis.

Il est essentiel de séparer les différentes formes d'aluminium en solution aqueuse dans la détermination de leur toxicité relative dans des lacs et cours d'eau acidifiés. Une brève extraction avec oxine à un pH de 8,3 sépare l'aluminium inorganique monomère et organique des formes polymères et amorphes. On peut éliminer la fraction organique par dialyse en équilibre avant extraction. Dans bien des cas, la fraction inorganique peut aussi être estimée à partir de mesures des fluorures libres et totaux. Les deux méthodes s'accordent bien dans des solutions artificielles (R^2 = 0,997) et assez bien dans des eaux naturelles (R^2 = 0,82), bien que les estimations calculées aient tendance à être légèrement inférieures aux valeurs mesurées directement. Lors de la fonte des neiges au printemps dans la région Muskoka–Haliburton du centre de l'Ontario, on a observé très peu d'aluminium polymère et amorphe, et la majeure partie de la fraction monomère inorganique se trouvait dans des complexes de fluorures. Je présente ici un modèle empirique permettant de prédire l'aluminium monomère inorganique dans ces eaux (R^2 = 0,90) à partir du carbone organique total et de l'aluminium mesurés par brève extraction sans dialyse.

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Acidic deposition releases large amounts of aluminum (Al) into streams and lakes in Scandinavia (Dickson 1980; Seip 1980; Seip et al. 1984), the northeastern United States (Johnson et al. 1981; Driscoll et al. 1983), and the Province of Quebec (Campbell et al. 1984). This Al is thought to be a primary causal factor in the decline of fish stocks in these areas (Schofield and Trojnar 1980; Muniz and Leivestad 1980). The exact mechanism by which Al exerts its toxic effect may vary depending on the organism and its life stage (Muniz and Leivestad 1980; Baker and Schofield 1982) but also may depend on which forms of Al dominate the ambient water chemistry (Driscoll et al. 1980. Organically complexed Al, for example, has been shown to be less toxic to brook trout (*Salvelinus fontinalis*) fry than the inorganic forms (Baker and Schofield 1982). However, part of the dificulty in quantitatively assessing the toxicity of Al, and particularly in comparing field studies with laboratory experiments, is that Al speciation in natural waters is very complex (Hem and Roberson 1967; Roberson and Hem 1969; Driscoll et al. 1983).

The primary intent of this paper is to describe, compare, and critically evaluate two methods for the speciation of Al in freshwaters. To accomplish this, I use both field and laboratory data and thus, secondarily, describe the amounts and forms of Al found during spring snowmelt in two acidified catchments in the Muskoka–Haliburton region of central Ontario. Fish kills in one of the catchments (Plastic Lake) during spring snowmelt have been documented by Harvey and Lee (1982).

Aluminum in freshwaters can exist as inorganic monomers (including Al^{3+}, $Al(OH)_x^{3-x}$, AlF_x^{3-x}, $Al(SO_4)_x^{3-2x}$), inorganic polymers, amorphous and microcrystalline Al, as well as organic forms. Several authors (Okura et al. 1962; Hem and Roberson 1967; Turner 1969; Smith 1971; Smith and Hem 1972) have shown that if the color development at pH 5 of the Al reagents oxine or ferron is measured rapidly enough, an estimate of inorganic monomeric Al can be obtained in the presence of polymeric forms. By allowing the reaction to proceed for 3–6 h, an estimate of polymeric and amorphous Al may also be obtained (Turner 1969). However, microcrystalline Al does not appear to react without substantial acidification (Hem and Roberson 1967; Turner 1971). These methods were all developed in simple artificial solutions without interferences.

In an attempt to apply these methods to natural freshwaters, Barnes (1975) introduced a pH shift to 8.3, which effectively eliminates fluoride complexing of monomeric Al, extracted and concentrated the Al–oxine complex into methyl isobutyl ketone (MIBK) to increase the sensitivity, and used atomic absorption spectroscopy to eliminate iron and other metal interferences. May et al. (1979)a) similarly extracted Al–oxine at pH 8.3 and noted that the pH shift to 8.3 could account for the relative lack of organic interference in highly colored waters. Others (Stary 1963; Lind and Hem 1975) have also shown that higher pH's can reduce the interference of many strong organic ligands. Neither Barnes (1975) nor May et al. (1979a) reported in detail the effect of an extraction at pH 8.3 on the kinetics of oxine's reaction with polymeric and amorphous Al.

I have slightly modified the Barnes (1975) extraction method as a measure of both inorganic monomeric and organic Al but have also applied equilibrium dialysis (Weber 1983) to separate organic Al from inorganic monomeric Al. A second method of estimating inorganic monomeric Al, based on fluoride measurements (Driscoll 1984), has also been evaluated and compared with the first. Other methods for the measurement and speciation of Al exist (Campbell et al. 1983; Seip et al. 1984; Driscoll 1984) but will not be evaluated here.

Laboratory Methods

One of the methods used in this comparison study is based on the Barnes (1975) procedure. In her original method, after the acid oxine (8-hydroxyquinoline) reagent is added, the samples are individually adjusted to a pH of approximately8.3, and then the buffer is added. This is time consuming and somewhat more difficult with small (3–5 mL) samples. As samples from this area of central Ontario are homogeneous and poorly buffered, enough excess NH_4OH is added to Barnes' ammonium acetate buffer to neutralize the additional acetic acid added with the oxine reagent, such that the final pH of the sample (buffer and oxine reagent added) is 8.3–8.8. To keep the contact time of the oxine with any polymers to the minimum, the buffer is first vigorously injected into the sample, the oxine reagent is added immediately afterwards, and MIBK is then added and the sample shaken. The whole procedure takes less than 15 s, giving what is operationally labelled "fast" reactive Al. The more slowly reacting polymers and amorphous Al are included when the buffer and oxine are allowed to react with the sample for a longer period before exraction (3–6 h depending on temperature, Turner 1969), giving "total" reactive Al.

The greater sensitivity of flameless atomic absorption spectroscopy (FAAS) allows smaller sample volumes to be extracted. The routine procedure is to put 3- to 5-mL sample volumes, 100 μL of the Barnes' buffer as modified, 100 μL of Barnes' oxine reagent, and 1.5 mL of MIBK into a polyallomer or polypropylene 15-mL centrifuge or culture tube. A rapid 7–10 s wrist-action, end-to-end shake follows. Then, 1 mL of the MIBK extract is pulled off and stored in a freezer until analysis by FAAS. When there are no possible intereferences, as in artificial solutions, the optical absorbance at 390 nm can be substituted for FAAS. If the test tubes are to be reused, they are washed three times in 95% ethanol and filled with 1% concentrated nitric acid until needed and then rinsed thoroughly with high-purity water just before use.

The dialysis procedure developed employs Spectrapor 6, 1000 molecular weight cutoff (mwco), 11.5-mm-diameter (3.5 surface to volume ratio) dialysis tubing. The tubing is initially cut to a length such that there is a 2–4% dialyzate to sample volume ratio. As there can be a fair amount of variation in the tubing diameter as supplied by the manufacturer, tubing with relatively small and large diameters should be discarded to maintain a uniform surface to volume ratio. The tubing is thoroughly rinsed with high-purity water and then soaked in 1% concentrated nitric acid for a minimum of 24 h. It is stored in this solution until needed, at which time the tubing is thoroughly rinsed in high-purity water, inside and out, and soaked for a minimum of 12 h and then rinsed and soaked for 12 h more in high-purity water. Just before use, the tubing is rinsed one more time, filled to capacity with high-purity water, clipped shut, rinsed, and placed into the sample container. Upon equilibration (see Results and Discussion: Dialysis Procedure), the tubing is retrieved, rocked gently to mix the contents, briefly rinsed in high-purity water, unclipped, and 3–5 mL of dialyzate removed. This dialyzate is subsequently analyzed for inorganic monomeric Al by the fast extraction procedure. The tubing is then rinsed and placed again in 1% concentrated nitric acid until needed. Disposable gloves are worn whenever handling the dialysis tubing to avoid contamination and to protect the tubing from puncture, and the tubing is always handled as gently as possible to avoid stretching or tearing. The overall sensitivity and precision of the technique has been evaluated on several occasions and has ranged from ±2 μg/L ±2% to ±6 μg/L ±12%, depending in part on the stability of the FAAS. A summary of the operational and functional definitions used in this procedure is given in Fig. 1.

For the free fluoride activities, we use an Orion fluoride selective electrode (model 94-09) at room temperature, the Nernstian response of which I have verified down to 100 nmol F/L in stirred samples without buffer (see also Baumann 1971). At the lower levels of free fluoride, we arbitrarily terminate the trace once drift drops below 0.1 mV/min (Barnard and Nordstrom 1982). An automated ion selective electrode method with a strong EDTA–citrate–phosphate buffer is used for total fluoride (S. Barnes, Ont. Minist. Environ., Rexdale, Ont., pers. comm.). The overall precision and sensitivity of these methods is considered under Results and Discussion. Total inorganic sulphate levels are measured by ion chromatography and corrected for $CaSO_4$ and $MgSO_4$ ion pairs using the equations in Table 1. The pH is measured against NBS buffers and corrected slightly for liquid junction potential differences.

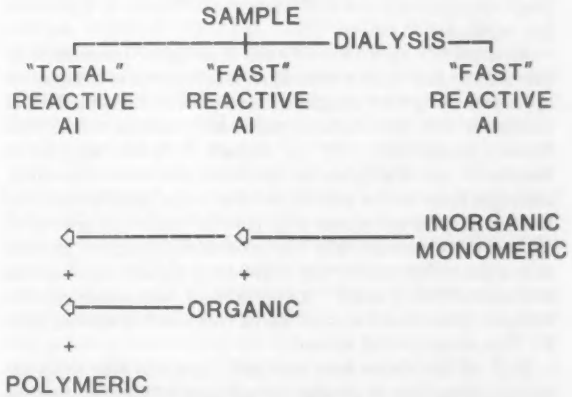

FIG. 1. Operational and functional definitions used in the extraction and dialysis procedures.

TABLE 1. Speciation formulas and association constants at 25°C.

Activity coefficient	Species = computational formula	$\log_{10}(B_i)$	Reference[a]
Y_0	$\{Al^{3+}\}$		
Y_1	$\{Al(OH)^{2+}\} = B_1\{Al^{3+}\}/\{H^+\}$	−4.99	1
Y_2	$\{Al(OH)_2^+\} = B_2\{Al^{3+}\}/\{H^+\}^2$	−10.13	1
Y_3	$\{Al(OH)_4^-\} = B_3\{Al^{3+}\}/\{H^+\}^4$	−22.16	1
Y_4	$\{AlF^{2+}\} = B_4\{Al^{3+}\}\{F^-\}$	7.01	2
Y_5	$\{AlF_2^+\} = B_5\{Al^{3+}\}\{F^-\}^2$	12.75	2
1	$\{AlF_3\} = B_6\{Al^{3+}\}\{F^-\}^3$	17.02	2
Y_7	$\{AlF_4^-\} = B_7\{Al^{3+}\}\{F^-\}^4$	19.72	2
Y_8	$\{AlF_5^{2-}\} = B_8\{Al^{3+}\}\{F^-\}^5$	20.91	2
Y_9	$\{Al(SO_4)^+\} = B_9\{Al^{3+}\}\{SO_4^{2-}\}$	3.2	2
Y_{10}	$\{Al(SO_4)_2^-\} = B_{10}\{Al^{3+}\}\{SO_4^{2-}\}^2$	5.1	2
1	$\{HF\} = B_{11}\{H^+\}\{F^-\}$	3.17	2
1	$\{CaSO_4\} = B_{12}\{Ca^{2+}\}\{SO_4^{2-}\}$	2.3	3
1	$\{MgSO_4\} = B_{13}\{Mg^{2+}\}\{SO_4^{2-}\}$	2.2	3
	$Ks = \{Al^{3+}\}\{OH^-\}^3$	32.65	4

[a]References: 1, May et al. 1979b; 2, Roberson and Hem 1969; 3, Plummer et al. 1976; 4, Hem and Roberson 1967.

Computational Methods

Three computational methods used in the speciation of inorganic monomeric Al are described. Given the estimate of inorganic monomeric Al concentration provided by the dialysis technique, the first and second methods further separate this into the concentrations of the various inorganic monomeric forms known to occur: $[Al^{3+}]$, $[Al(OH)_x^{3-x}]$, $[AlF_x^{3-x}]$, and $[Al(SO_4)_x^{3-2x}]$. When free fluoride, $\{F^+\}$, activities are available, Al^{3+} activities, $\{Al^{3+}\}$, can be estimated as

$$(1)\quad \{Al^{3+}\} = [\text{inorganic monomeric Al}]/(1/Y_0 + C_1/\{H^+\} + C_2/\{H^+\}^2 + C_3/\{H^+\}^4 + C_4\{F^-\} + C_5\{F^-\}^2 + C_6\{F^-\}^3 + C_7\{F^-\}^4 + C_8\{F^-\}^5 + C_9\{SO_4^{2-}\} + C_{10}\{SO_4^{2-}\}^2)$$

where $C_i = B_i/Y_i$, B_i is the association constant, Y_i is the activity coefficient, and i is the species as listed in Table 1. Once $\{Al^{3+}\}$ has been obtained, all the other inorganic monomeric species can be calculated using the formulae in Table 1. The extended Debye–Hückel formula for activity coefficients is applicable to the uniformly dilute freshwaters studied here (ionic strengths are 4×10^{-4} to 6×10^{-4} mol/L). This is the first method.

Alternatively, given the estimate of inorganic monomeric Al provided by the dialysis technique, it is possible to compute its speciation using total inorganic fluoride rather than free fluoride estimates; but this method necessarily assumes that total fluoride equals $[HF] + [F^-] + \Sigma x[AlF_x^{3-x}]$. Ca, Mg, and Fe complexes are negligible in the dilute waters studied here, although there is the possibility that some measurable total fluoride may be associated with polymeric and amorphous Al (Hem and Roberson 1967). The procedure is iterative, quickly converges within two to four steps, and provides simultaneous estimates of $\{Al^{3+}\}$ and $\{F^-\}$ (Appendix A), from which all other inorganic monomeric species can be computed as before (Table 1). This is the second method.

Both of the above two speciation methods rely upon the dialysis procedure to provide an estimate of inorganic monomeric Al, from which $\{Al^{3+}\}$ is initially computed (along with $\{F^-\}$ in the second procedure). Driscoll (1984) has suggested an alternative method that eliminates the direct measurement of inorganic monomeric Al, requiring only free and total fluoride (and pH, etc.). The necessary assumption is again that total fluoride consists only of $[HF] + [F^-] + \Sigma x[AlF_x^{3-x}]$, which permits $\{Al^{3+}\}$ to be obtained from

$$(2)\quad \{Al^{3+}\} = ([\text{Total F}] - [HF] - [F^-])/(C_4\{F^-\} + 2C_5\{F^-\}^2 + 3C_6\{F^-\}^3 + 4C_7\{F^-\}^4 + 5C_8\{F^-\}^5)$$

where [HF] can be obtained from the equation in Table 1. As before, using $\{Al^{3+}\}$, the other inorganic monomeric forms of Al can be computed, and if their sum is taken, an estimate of inorganic monomeric Al is obtained that is completely independent of the dialysis procedure. The precision and sensitivity of this estimate of inorganic monomeric Al is good only when the ALF_x^{3-x} species dominate total fluoride, as will be shown under Results and Discussion.

All of these calculations are based on association constants at 25°C, which is close to the temperatures used for the free fluoride and the dialysis procedures (see Results and Discussion); the actual speciation of field samples will vary somewhat from this at field temperatures.

Results and Discussion

Extraction Procedure

By adding the buffer first, sufficient time is allowed for the dissociation of ALF_x^{3-x} complexes before extraction, even with the fast (<15 s) procedure. I have obtained a range in recovery from 99 to 101% with up to 100/μg F/L and 0–800 μgAl/L, similar to the results of Barnes (1975) and May et al. (1979a).

Turner and Sulaiman (1971) note that solution pH affects the rate of polymeric and amorphous Al decomposition in oxine solutions. Consequently, it was necessary to verify that a 15-s extraction at pH 8.3 was sufficient to exclude most polymers, as is the case at pH 5 (Turner 1969). To accomplish this, an acid 500 μgAl/L stock solution was slowly neutralized with NH_4OH to pH 5.3 and allowed to stand for 2 wk, after which the final pH was 5.0. Buffer and oxine reagents were added to obtain the two final pH's, and aliquots were then extracted at different times. Despite the extent of polymerization, there was little difference in estimated Al (micrograms per litre, mean ± SD, $N = 2$) between the two reaction pH's at any time:

Reaction time	Reaction pH 5.0	Reaction pH 8.3
15 s	261 ± 10	270 ± 10
3 h	330 ± 5	320 ± 10
6 h	390 ± 10	400

Al standards and blanks that are kept acid (pH 3) before the addition of reagents exhibit a constant absorbance over time at both reaction pH's.

The kinetic response of oxine at pH 8.3 to another neutralized 500 μgAl/L solution (aged only 24 h, final pH 4.7) was then compared with a natural water sample (pH 4.6–4.8) from the Muskoka–Haliburton region of Ontario (Fig. 2). The results suggest that a fast (15 s) extraction does provide a reasonable and easy separation between inorganic monomeric and polymeric Al both in neutralized artificial Al solutions (Turner 1969)

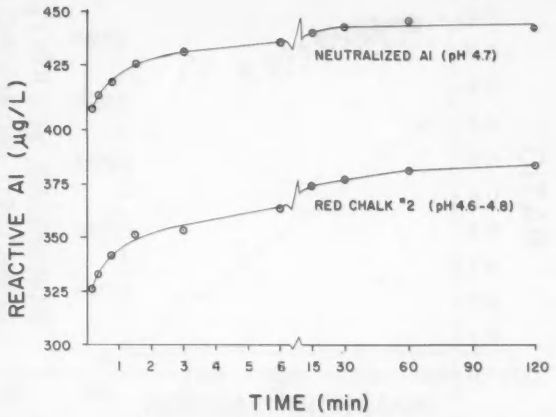

FIG. 2. Amount of Al that reacts with oxine at pH 8.3 over time in a rapidly neutralized standard solution and in a natural water sample.

and in natural waters. For the most precise work, an extrapolation to zero time may be required (Smith 1971).

To verify that organic Al is extracted by this procedure, standards containing between 0 and 500 µgAl/L were spiked with approximately 250 µmol salicylic acid/L and allowed to stand over night. According to Gamble et al. (1970), the strongest ligand group available on fulvic acids is a salicylic acid group with one hydroxyl ortho to the carboxyl group. For soil fulvic acids, they estimated that there are only 3.3 µmol of salicylic acid groups per milligram of carbon of fulvic acid, whereas Oliver et al. (1983) have shown that there are, on the average, 10 µmol of carboxyl groups in all configurations per milligram of carbon of freshwater fulvic acid. If we assume that all these 10 µmol/mg C of carboxyl groups are in the salicylic acid configuration, then 25 mg C/L of fulvic acid would provide 250 µmol/L of salicylic acid groups. This is obviously a worst-case scenario; nonetheless, 98–101% recovery of Al was obtained under these conditions. Hoyt and Turner (1975) recovered only 80% Al with oxine at pH 5 and an unreported amount of salicylic acid and Al present. They also report interference in aqueous alfalfa meal extracts, but the amount of organic carbon in these extracts was probably very large. This suggests that interference can occur but probably not under the conditions found in natural waters when a reaction pH of 8.3 or above is used. In addition, the similarity of the reaction kinetics of a natural-colored freshwater sample with that of an artificially polymerized Al solution (Fig. 2) is consistent with the hypothesis that it is polymeric and amorphous Al and not organic Al that is responsible for slowly reacting Al in these natural waters.

In general, I conclude that the fast (15 s) extraction method adequately measures inorganic monomeric and organic Al. In the presence of large amounts of rapidly reacting polymeric Al, there may be some overestimation whereas in the presence of unreactive organic Al there may be some underestimation. In addition, a longer reaction time with oxine (3–6 h depending on temperature, Turner 1969) may be used to estimate polymeric and amorphous Al, although there is some possibility that slowly reacting organic Al (e.g. Campbell et al. 1983), if present, would be included in this estimate.

Dialysis Procedure

Given the estimate of Al provided by the fast extraction method, it is next necessary to separate it into the organic and inorganic monomeric fractions. Equilibrium dialysis is one method for obtaining this separation (Benes and Steinnes 1974; Truitt and Weber 1981; Weber 1983). It is usually accomplished by suspending dialysis tubing filled with high-purity water in a much larger volume of the sample to be analyzed. As the molecular weight of freshwater fulvic acid can range from 500 to 3000 (Reuter and Perdue 1977; Dawson et al. 1981), dialysis tubing with the smallest available pore size should be employed. However, even 1000-mwco tubing will permit the entry of smaller fulvics and Al–fulvics over a long period of time. For example, when measuring organic penetration by its absorbance at 330 nm, I routinely find that 6%, and occasionally up to 12%, of the absorbing material will penetrate a 1000-mwco membrane within the time period required for equilibrium dialysis of the inorganic fraction. Whether this material represents metal–organic complexes has not been determined, but it does suggest that minimizing the dialysis time, especially in organic-rich water, would be a reasonable precaution against overestimation of the inorganic monomeric fraction of Al. Minimizing the dialysis time is also of interest in preserving sample integrity.

The rate at which solutes approach equilibrium inside dialysis tubing depends on the surface to volume ratio and the membrane's permeability to the solute, which in turn is a function of temperature and effective pore size. In addition, for charged species, the rate at which the dominant co-diffusing ion of opposite charge can permeate the tubing exerts a considerable effect if it is much slower than the specific ion of interest. Assuming that the volumes inside and outside the dialysis tubing are well mixed and the inside volume is much less than the outside:

$$(3) \qquad C_{in}/C_{out} = 1 - e^{-kt}$$

where C is the concentration of solute (inside or outside of the dialysis tubing) and t is time (hours). For any given solute, k is a function of $P(A/V)$, where P (centimetres per hour) is the permeability of the dialysis tubing and A/V is the surface to volume ratio. As A/V increases (i.e. as the diameter of the cylindrical tubing decreases), k increases and equilibrium is approached more rapidly. The equilibration rate is not only a function of temperature but of the co-diffusing ion present (Fig. 3, compare the Al^{3+}, Cl^- pair with the Al^{3+}, SO_4^{2-} pair). Apparently, the equilibration of cations in acidified freshwater is inhibited by the slow equilibration of sulphate. Temperature may also exert its effect on sulphate and only secondarily on the cations. I conclude (from Fig. 3 and equation 3) that after a 24-h dialysis with no stirring at 20–25°C, 90–95% of the inorganic monomeric Al should enter the dialysis tubing that I used (Spectrapor 6, 1000 mwco, nominal diameter 11.5 mm). Any further dialysis time will only increase this percentage slightly while permitting more organic Al to enter. Alternatively, longer periods at lower temperatures might be preferable. The replicability of the dialysis procedure (when sampled at equilibrium) followed by a fast extraction has been measured on several occasions with standard deviations varying from 2 to 12% of the mean. The relatively poor replicability observed in parts of Fig. 3 occurs because (i) when sampling before equilibrium has been approached, the natural variations in dialysis tubing diameter and pore size have a greater effect on the estimate of k, (ii) when sampling close to equilibrium, C_{in} becomes similar to C_{out}, making the estimate of k more prone to error, and (iii) the assumptions underlying equation 3 do not exactly hold.

When equilibrium dialysis is used in highly colored, dilute

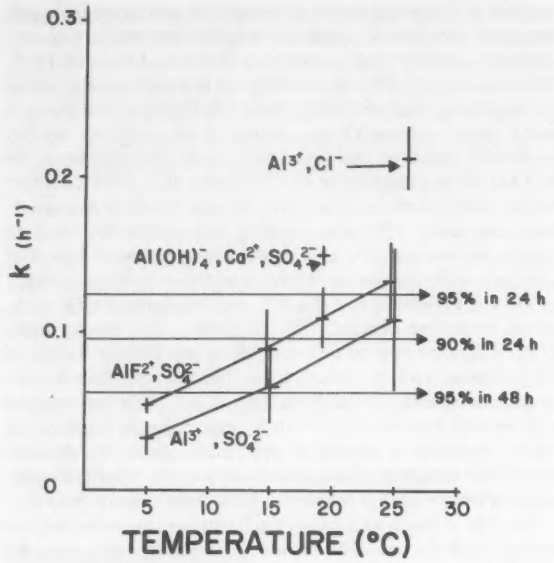

FIG. 3. Value of $k \pm 95\%$ C.I. from $C_{in}/C_{out} = 1 - e^{-kt}$, where C is the concentration of the Al species inside or outside the dialysis tubing for different temperatures, dominant co-diffusing inorganic anions, and with no agitation. The solutions for which SO_4^{2-} was the dominant anion were designed to simulate natural acidified freshwaters with an ionic strength of 5.3×10^{-4} mol/L and with Ca^{2+}, Mg^{2+}, and H^+ as the dominant cations. Total Al and fluoride (when present) ranged from 100 to 300 µg/L; pH was less than 4.5 or greater than 8.5 (for $Al(OH)_4^-$).

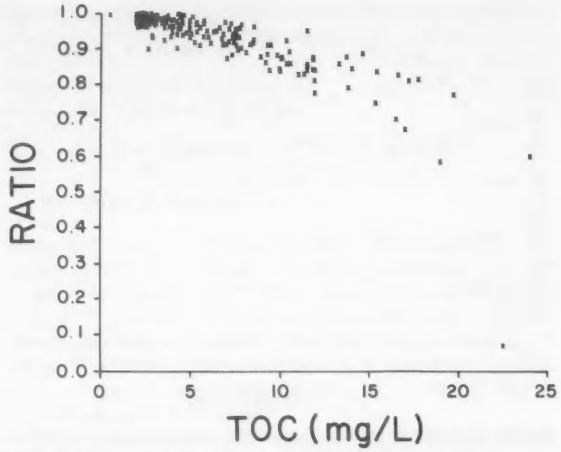

FIG. 4. Ratio of measured to Donnan-corrected inorganic monomeric Al versus total organic carbon (TOC) in streams and lakes of the Muskoka–Haliburton region of central Ontario during spring snowmelt, 1982.

waters, Donnan disequilibrium can occur if the organic anions make up a substantial fraction of the total equivalents of anions (Bull 1951). In this circumstance there will be more inorganic anions inside and more cations outside the dialysis tubing at equilibrium than would normally occur. Knowing the relative proportion of monovalent and divalent inorganic anions and cations, a correction factor can be calculated (Appendix B) and applied to the inorganic monomeric Al found inside the dialysis tubing. The accuracy of this procedure depends on the estimates of the sums of inorganic mono- and di-valent ions. For example, if "total" calcium and magnesium estimates are used, assuming no organic association, then the influence of the organics on Donnan disequilibrium will be overestimated. Another more random source of error is analytical, as several independent measurements are used to obtain the sums of inorganic mono- and di-valent ions. In general, the use of these corrections can leads to the magnification of errors in the inorganic monomeric Al estimates and thus should be treated with caution. In addition, as the amount of organic Al passing through the dialysis tubing may be greater in organic-rich water, some cancellation of Donnan error may occur. I do not routinely apply Donnan corrections but am still evaluating their utility; in certain circumstances their application may be necessary (Fig. 4).

Adsorption of Al onto the dialysis tubing and container walls during equilibration and any additional sample instability over time are potential problems with the dialysis technique (Weber 1983). In the artificial solutions I used (Fig. 3; which approached acidified natural freshwaters in composition) no losses greater than 5% were found. In actual freshwater samples, small changes have been occasionally observed in free fluoride levels after bringing the samples to room temperature. This may reflect losses of inorganic monomeric Al equivalent to 2–7 µg/L over 10 d. However, no significant differences were observed in free fluoride after 1 d of storage with or without dialysis tubing in three different freshwater samples. Benes and Steinnes (1974) have also found that there is little problem with adsorption of unsaturated Al solutions to dialysis tubing. It is unclear, then, whether the changes I observed represent adsorption to the container walls or disequilibrium between inorganic monomeric Al and polymeric or organic Al, perhaps induced by the shift to laboratory temperatures (Seip et al. 1984). Whatever the causes and although small, they provide further motivation for a minimum equilibration time during dialysis.

A second attempt at the physical separation of organic Al from inorganic monomeric Al was carried out with an Amicon ultrafiltration apparatus and Amicon UM-2 ultrafilters. However, large amounts of Al adsorbed onto the surface of these filters even at pH 3 and when background electrolytes were added. As Benes and Steinnes (1974) and C.T. Driscoll (Dep. Civ. Eng., Syracuse University, Syracuse, NY, pers. comm.) have made similar observations, the method was not pursued further.

Computational Procedure and Field Comparison

To provide an integrated test of the computational methods, as well as the free fluoride and pH measurements, I adjusted artificial solutions of background electrolytes (as used in Fig. 3) plus 150 µgF/L at pH 4.1 to Al concentrations ranging from 50 to 500 µg/L. Free fluoride was then measured as described (reported as $pF = -\log_{10}\{F^-\}$) and compared with that calculated by the second method described under Computational Methods and in Appendix A. The experimental range of pF's was similar to that encountered locally in natural waters (5.25–7.10). There was no difference between the calculated and measured pF's, and they were highly correlated ($pF_{calc} = -0.1 + 1.03pF_{meas}$; $R^2 = 0.999$; slope not significantly different from 1.00 at 95% confidence). When, however, the calculated inorganic monomeric Al (using the third method

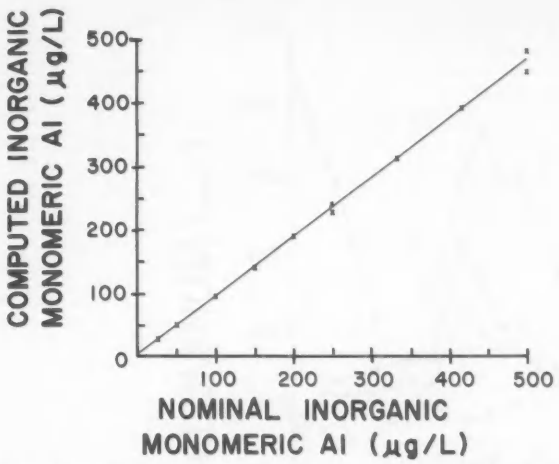

FIG. 5. Comparison of computed versus nominal inorganic monomeric Al in artificial solutions. Background electrolytes as in Fig. 3. $[Al]_{calc} = 4 + 0.93\ [Al]_{add}$, µg/L; $R^2 = 0.997$.

described under Computational Methods) was compared with the nominal Al additions (Fig. 5), it was systematically less by 7% but also highly correlated ($[Al]_{calc} = 4 + 0.93[Al]_{add}$; $R^2 = 0.997$; slope significantly different from 1.00 at 95% confidence). At present, I have not determined the source of this error; nonetheless, the experiment shows that the computational methods can be very precise and well within 10% of the expected values in acidic inorganic Al solutions.

The computational method for estimating inorganic monomeric Al does, however, fail at higher pH's. As the AlF_x^{3-x} complexes make up a small proportion of total fluoride at the higher pH's, small errors in the free and total fluoride measurements are magnified into extreme deviations in computed inorganic monomeric Al values. This is illustrated in a sensitivity analysis of the method using three natural water samples in Table 2. At low pH's, a 10% error in free or total fluoride or pH results in a worst-case error of about 10% in the computed inorganic monomeric Al. At pH 5.5, however, a 10% error in either the free or total fluoride measurements would result in almost a 70% change in the computed value. At even higher pH's, extremely large *negative* values for inorganic monomeric Al can be obtained if the free fluoride estimate is only insignificantly larger than total fluoride. In general, the computational method should only be employed at pH's less than 5.5 and with caution between 5.0 and 5.5, unless there are large enough amounts of inorganic monomeric Al to ensure that the AlF_x^{3-x} complexes make up a significant proportion of total fluoride.

The field estimates of inorganic monomeric Al from the dialysis technique are similar to those obtained using the free–total fluoride method of computation, when only those values for pH < 5.5 are considered (Fig. 6). The two methods are well correlated ($R^2 = 0.82$) but there is, nonetheless, substantially more unexplained variance than obtained with the artificial solutions (Fig. 5). In testing the hypothesis that the slope of the regression between the two methods equals 1, the result depends on which variable is assumed to be the independent, error-free variable; if the computed estimate is chosen, then the slope is not significantly different from 1.00 and the intercept is not significantly different from 0.0 at the

TABLE 2. Sensitivity analysis of computed inorganic monomeric Al (µg/L ± % error from nominal value).

pH	5.50	4.71	4.48
pF	5.74	6.74	7.02
TF (µg/L)	41.5	60.5	63.4
Nominal Al	7.30	150	224
$[H^+]$ + 10%	7.05 (−3%)	147 (−2%)	221 (−1%)
$[H^+]$ − 10%	7.63 (+4%)	154 (+3%)	228 (+7%)
$[F^-]$ + 10%	2.86 (−61%)	141 (−6%)	209 (−7%)
$[F^-]$ − 10%	12.4 (+69%)	161 (+7%)	243 (+8%)
[TF] + 10%	12.4 (+69%)	166 (+11%)	247 (+10%)
[TF] − 10%	2.23 (−69%)	134 (−11%)	201 (−10%)

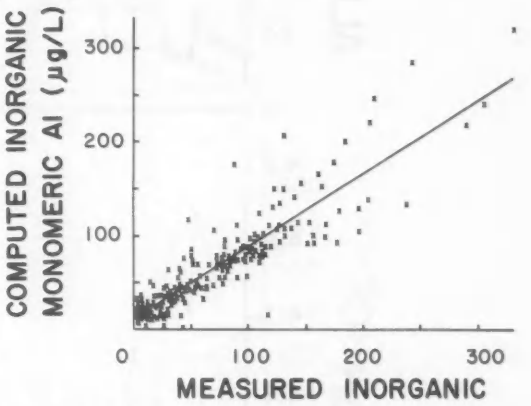

FIG. 6. Comparison of computed versus measured inorganic monomeric Al in streams and lakes of the Muskoka–Haliburton region of central Ontario during spring snowmelt, 1982. $[Al]_{calc} = 11 + 0.78\ [Al]_{meas}$, µg/L; $R^2 = 0.82$.

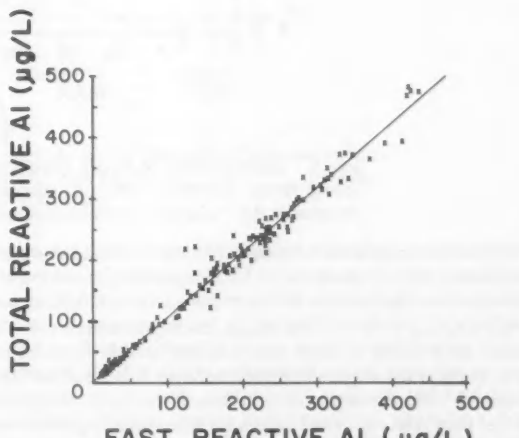

FIG. 7. Comparison of Al reacting with oxine at pH 8.3 within 15 s ("fast") versus that reacting after 3–6 h ("total") in streams and lakes of the Muskoka–Haliburton region of central Ontario during spring snowmelt, 1982. $[Al]_{tot} = 2 + 1.06\ [Al]_{fast}$, µg/L; $R^2 = 0.987$.

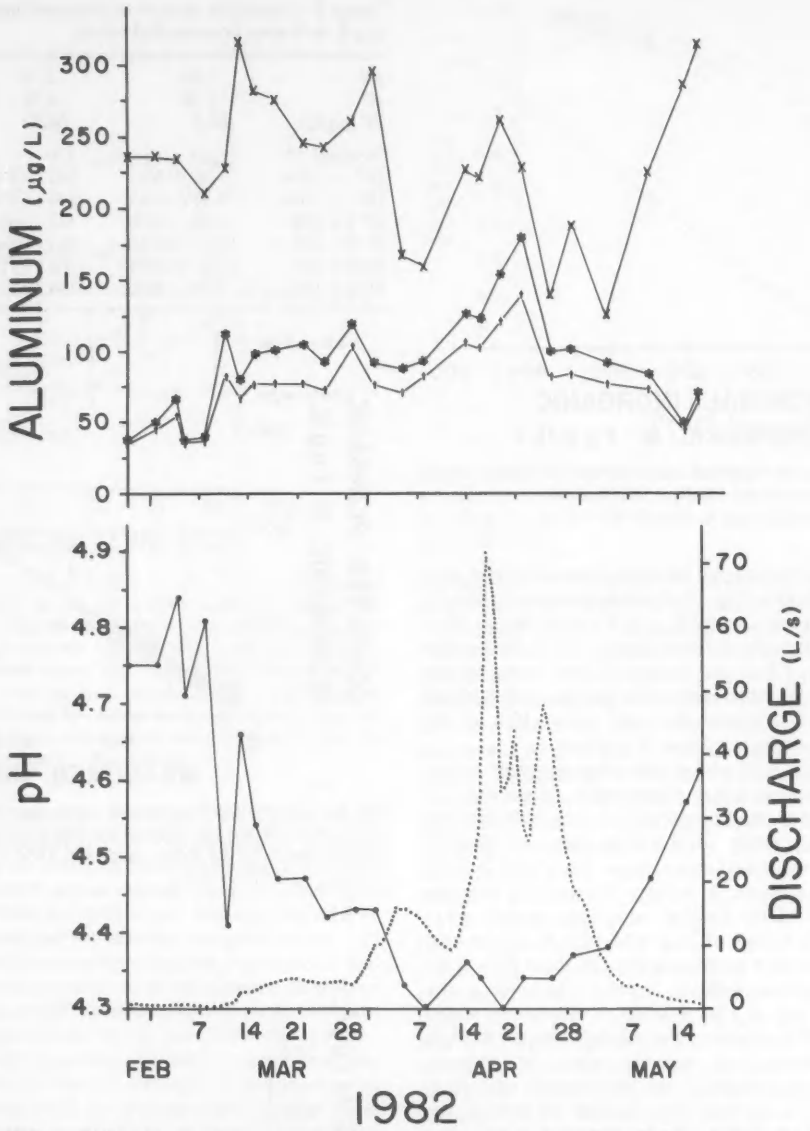

FIG. 8. Different forms of Al, pH, and discharge in Plastic Lake inflow (Muskoka–Haliburton region) during spring snowmelt, 1982. ×, inorganic monomeric plus organic Al ("fast"); *, inorganic monomeric Al; +, ΣAlF_x^{3-x}. The dotted line represents discharge.

95% level of confidence whereas when the measured inorganic monomeric Al is considered to be independent, the slope and intercept are significantly different from 1.00 and 0.0, respectively ($[Al]_{calc} = 11 + 0.78[Al]_{meas}$, micrograms per litre). The actual relationship is most likely somewhere in between the two. Perhaps the major difference between this result and that obtained with the artificial solutions is in the <50 µgAl/L range. Here, the computed values are substantially greater than the measured inorganic Al, which tends to lever the regression to a higher intercept and lower slope. The reasons for this discrepancy are unclear; the computational method is, however, extremely sensitive to systematic errors when the $[\Sigma xAlF_x^{3-x}]$ levels are low. Because of the low environmental levels of polymeric and amorphous Al present (see Field Results), fluoride associated with these forms and measured in the total fluoride procedure would not be expected to interfere substantially with the computational method. If the measured inorganic monomeric Al is corrected for the Donnan effect (Appendix B) there is a decrease in the correlation coefficient as well as the slope.

Field Results

Using the analytical methods described, I conducted a study of Al speciation in several streams and lakes of the Muskoka–Haliburton area of central Ontario during spring snowmelt

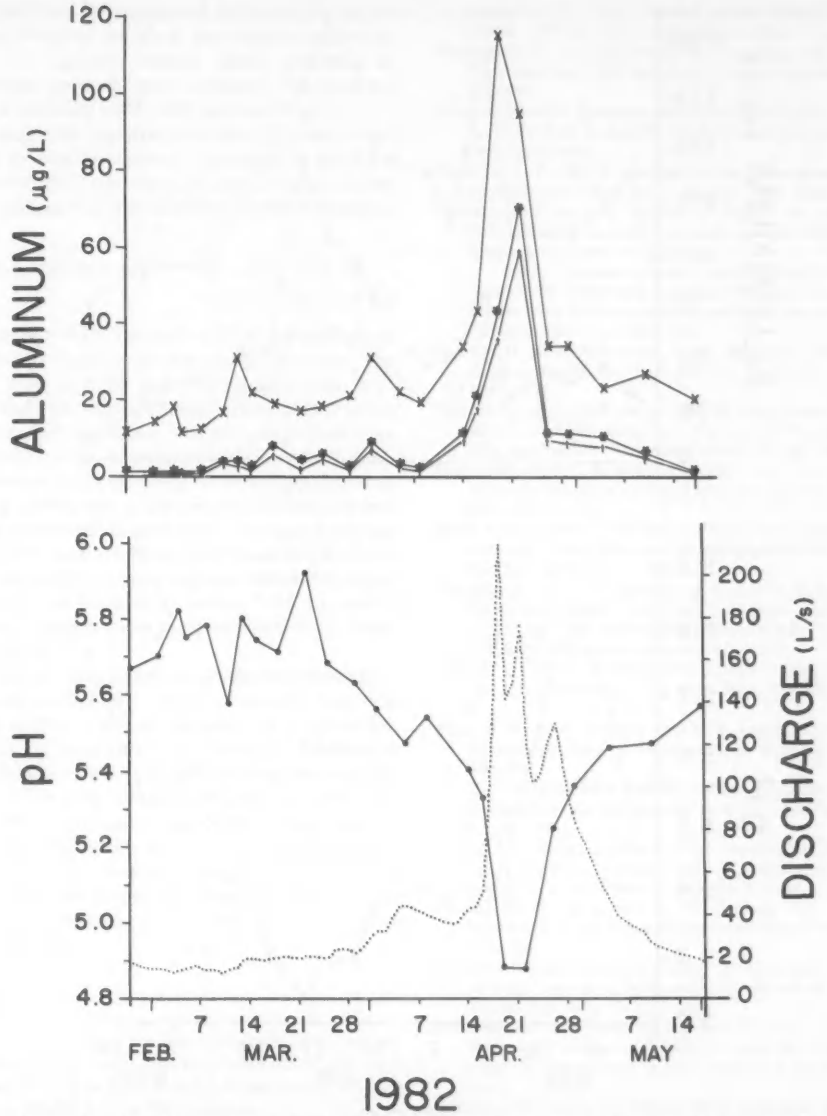

FIG. 9. Different forms of Al, pH, and discharge in Plastic Lake outflow (Muskoka–Haliburton region) during spring snowmelt, 1982. ×, inorganic monomeric plus organic Al ("fast"); *, inorganic monomeric Al; +, ΣAlF_x^{3-x}. The dotted line represents discharge.

(March–May) of 1982. During this period, total reactive Al (up to 3–6 h reaction time with oxine) and fast-reacting Al (<15 s) were highly correlated ($R^2 = 0.987$) (Fig. 7), with an average of only 6% more total than fast-reacting Al ($[Al]_{total} = 2.0 + 1.06[Al]_{fast}$, micrograms per litre). I conclude that, on the average, polymeric and amorphous Al in suspension is not important in these waters during spring snowmelt, a conclusion that is substantiated by the fact that none of these samples were saturated with respect to microcrystalline gibbsite at 25°C (Table 1; Hem and Roberson 1967). At the colder field temperatures, the samples would be further from saturation (Seip et al. 1984). Hem and Roberson's (1967) solubility product for microcrystalline gibbsite was determined from supersaturation in neutralized polymeric solutions, thus simulating what might be expected to occur under field conditions when acidified, Al-rich water is neutralized. That I was not able to observe supersaturation under field conditions or precipitation in artificially mixed lake and stream waters during spring snowmelt (B. D. LaZerte, unpubl. data) suggests that surface adsorption of Al onto particulates, perhaps obtained from suspended sediments during spring turnover, might be a more important mechanism for Al losses in lakes of this area. However, as flow rates drop in late summer, these streams often become more neutralized and higher proportions of polymeric Al have been found (B. D. LaZerte, unpubl. data).

It follows that Al toxicity observed in this area during spring

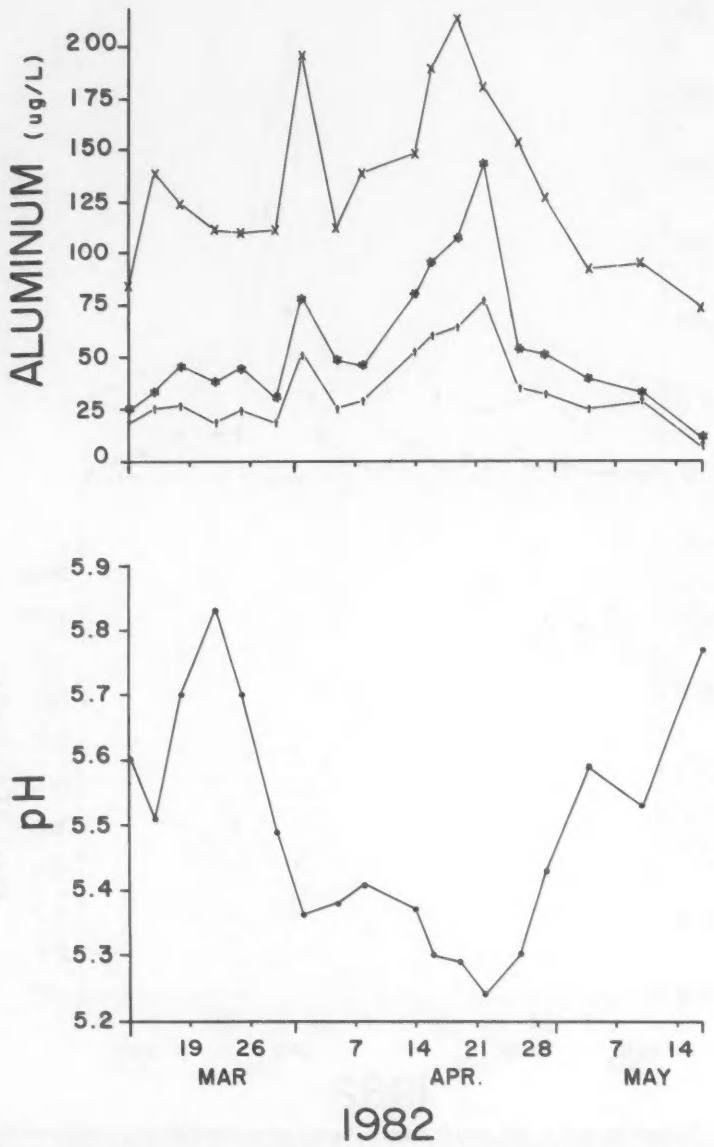

FIG. 10. Different forms of Al and pH in Paint Lake inflow (Muskoka–Haliburton region) during spring snowmelt, 1982. ×, inorganic monomeric plus organic Al ("fast"); *, inorganic monomeric Al; +, ΣAlF_x^{3-x}.

snowmelt (Harvey nd Lee 1982; K. L. Clark and R. J. Hall, unpubl. data) is more likely the result of the much more abundant inorganic monomeric forms rather than polymeric and amorphous Al. Toxicity experiments designed to simulate field conditions in this area should, then, avoid polymerized solutions supersaturated with respect to microcrystalline gibbsite; also, previously reported toxicity studies performed under supersaturating conditions (e.g. parts of Muniz and Leivestad 1980; Schofield and Trojnar 1980; Baker and Schofield 1982) may not be relevant to field toxicity in this area.

The different forms of Al present during spring snowmelt vary dramatically as a function of pH, organic carbon, and flow rates (Fig. 8, 9, 10). In general, the importance of the organic complex in colored waters and the importance of the fluoride complex to inorganic monomeric Al is confirmed (Johnson et al. 1981; Driscoll et al. 1983; Campbell et al. 1984; Seip et al. 1984). The importance of the fluoride complex suggests that more detailed attention be paid to its toxicity (e.g. Baker and Schofield 1982; K. L. Clark and R. J. Hall, unpubl. data). A good illustration as to how even highly colored streams can switch from primarily organically complexed to inorganically complexed monomeric Al during peak flow can be seen in Fig. 8. As it is the inorganic fraction that is potentially most toxic (Baker and Schofield 1982), this switch could have important

ecological consequences. To compound the problem, at this time the incoming streams can flush the shallow under-ice, littoral areas (B. D. LaZerte, unpubl. data), important as feeding and spawning grounds, and overwhelm the chemical composition of the normally stable lake outflow (Fig. 9).

Because of the potential importance of the inorganic monomeric form of Al to toxicity studies, I attempted to predict it solely from the more easily measured pH, total organic carbon (TOC), and fast-reacting Al. The following empirical equation was obtained:

$$(4) \quad [\text{Inorganic monomeric Al}] = -10 + 0.69\,[\text{fast Al}] - 0.03\,[\text{TOC}][\text{fast Al}]$$

with $R^2 = 0.90$ and $N = 537$. The units of TOC are milligrams per litre and for the Al fractions are micrograms per litre. The pH ranged from 4.09 to 6.91 and TOC ranged from 1 to 24 mg/L. The pH and TOC alone contributed little to the overall regression and were thus discarded. The pH was probably not significant because of its high correlation with fast-reacting Al. This regression has not yet been tested on an independent data set and thus strictly applies only to the streams and lakes studied here during spring snowmelt. In particular, it will probably not predict well in the very clear, high-Al lakes found in extremely acidified regions, as data from these regions were not included in the analysis. This can be seen by setting TOC to zero in equation 4 and then noting that inorganic monomeric Al is only 69% of fast-reacting Al.

In summary, both the dialysis and computational methods of estimating inorganic monomeric Al are consistent and well correlated with each other. The general pattern of Al speciation observed during spring snowmelt in the Muskoka–Haliburton region of central Ontario agrees well with previous observations by Driscoll et al. (1983) in the Adirondack region of New York, Campbell et al. (1983) in the Laurentians of Quebec, and Seip et al. (1984) in Norway. The results are relevant to the interpretation of observed field toxicity and in relating laboratory studies to the field. The empirical model provided may be useful in estimating the potentially toxic Al fraction in colored, acid waters of central Ontario.

Acknowledgments

This work would not have been possible without the assistance of Chuck Cox, Dave Sturgis, and many other members of the Limnology Section of the Water Researces Branch and the Laboratory Services Branch of the Ontario Ministry of the Environment.

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Appendix A

Given the concentration of inorganic monomeric Al, [TA], and total F, [TF], an initial estimate of $\{Al^{3+}\}$ and $\{F^-\}$ is obtained by assuming that $[TF] = [AlF^{2+}] + [HF] + [F^-]$ only. This is actually very close in many cases, as AlF_2^+ is the only other F complex of any consequence in these waters. Thus, two equations in two unknowns can be set up and solved directly:

$$(A.1)\quad [TA] = \{Al^{3+}\}(1/Y_0 + C_1/\{H^+\} + C_2/\{H^+\}^2 + C_3/\{H^+\}^4 + C_4\{F^-\} + C_9\{SO_4^{2-}\} + C_{10}\{SO_4^{2-}\}^2)$$

$$(A.2)\quad [TF] = \{F^-\}(1/Y_F + C_{11}\{H^+\} + C_4\{Al^{3+}\}).$$

The solutions obtained, $\{Al^{3+}\}_i$ and $\{F^-\}_i$, can then be substituted into the full equations for inorganic monomeric Al and total fluoride, including *all* the AlF_x^{3-x} species, to obtain calculated initial estimates of $[TA]_i$ and $[TF]_i$. When these are compared with the observed values, the following differentials are obtained:

$$(A.3)\quad dTA_i = [TA] - [TA]_i$$

$$(A.4)\quad dTF_i = [TF] - [TF]_i.$$

The partial derivatives of the *full* equations for TA and TF can also be obtained and the initial estimates, $\{Al^{3+}\}_i$ and $\{F^-\}_i$, substituted into them giving

$$\partial TA/\,d\{Al^{3+}\}\,|_i,\ \partial TF/\,d\{Al^{3+}\}\,|_i,$$
$$\partial TA/\,d\{F^-\}\,|_i,\ \text{and}\ \partial TF/\,d\{F^-\}\,|_i.$$

The appropriate corrections for $\{Al^{3+}\}_i$ and $\{F^-\}_i$ are then found by solving

$$(A.5)\quad \begin{bmatrix} d\{Al^{3+}\} \\ d\{F^-\} \end{bmatrix}_i = \begin{bmatrix} dTA/\,d\{Al^{3+}\} & dTA/\,d\{F^-\} \\ dTF/\,d\{Al^{3+}\} & dTF/\,d\{F^-\} \end{bmatrix}_i^{-1} \begin{bmatrix} dTA \\ dTF \end{bmatrix}_i.$$

The new, corrected approximations become

$$(A.6)\quad \{Al^{3+}\}_{i+1} = \{Al^{3+}\}_i + d\{Al^{3+}\}_i$$

$$(A.7)\quad \{F^-\}_{i+1} = \{F^-\}_i + d\{F^-\}_i.$$

These new approximations are then substituted back into the full equations, as before, and the process is repeated as many times as is necessary until $[TA]_{i+j}$ and $[TF]_{i+j}$ are close enough to the observed values and/or there is little difference between successive estimates of $\{Al^{3+}\}$ and $\{F^-\}$. Using the criterion that successive estimates should be less than 0.1% different, I routinely find that two to four iterations are all that is necessary.

Appendix B

If the volume inside the dialysis tubing is negligible relative to that outside, then at equilibrium (Bull 1951):

$$(B.1)\quad ([C_{out}^{x+}]/[C_{in}^{x+}])^{1/x} = ([A_{in}^{x-}]/[A_{out}^{x-}])^{1/x} = R$$

where [C] and [A] represent equivalents of individual cations and inorganic anions, respectively, and x is their charge. Charge balance is required inside the dialysis tubing and thus, assuming only mono- and di-valent inorganic ions,

$$(B.2)\quad \Sigma[C_{in}^+] + \Sigma[C_{in}^{2+}] = \Sigma[A_{in}^-] + \Sigma[A_{in}^{2-}].$$

Rearranging B.1 to solve for the inside ions and using these in B.2 gives, eventually,

$$(B.3)\quad R^4\Sigma[A_{out}^{2+}] + R^3\Sigma[A_{out}^+] - R\Sigma[C_{out}^+] - \Sigma[C_{out}^{2+}] = 0$$

which can be solved exactly for R, knowing the sums of inorganic monovalent and divalent cations and anions. R, R^2, and R^3 are then calculated and applied to the various inorganic monomeric Al species (using B.1) initially calculated; this provides a corrected value of inorganic monomeric Al from which the individual species are recalculated.

Mollusca of Six Low-Alkalinity Lakes in Ontario

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Rooke, J. B., and G. L. Mackie. 1984. Mollusca of six low-alkalinity lakes in Ontario. Can. J. Fish. Aquat. Sci. 41: 777–782.

In 1980 and 1981, we sampled the molluscs of six Ontario lakes with low alkalinity concentrations. Contrary to previous reports of rarity or absence of molluscs in low-alkalinity waters, we showed that there was no consistent difference in the number of species present in these lakes, across a limited range of alkalinity (0.24–3.17 $mg \cdot L^{-1}$). It is possible that molluscs are more dense in lower alkalinity lakes, due primarily to large numbers of *Pisidium casertanum* and *P. ferrugineum* found there. Significant differences between mollusc densities in two intermediate-alkalinity lakes indicate that factors other than alkalinity may have affected mollusc distributions. The unionid clam, *Elliptio complanata*, was found in two lakes with mean alkalinities of 0.41 $mg \cdot L^{-1}$.

En 1980 et 1981, nous avons prélevé des échantillons de mollusques de six lacs de l'Ontario à faible teneur en alcalinité. Contrairement à des rapports antérieurs à l'effet que les mollusques sont rares ou absents dans des eaux de faible alcalinité, nous démontrons qu'il n'y a pas de différence uniforme du nombre des espèces présentes dans ces lacs, dans une gamme limitée d'alcalinités (0,24–3,17 $mg \cdot L^{-1}$). Les mollusques sont possiblement plus denses dans les lacs de faible alcalinité, surtout à cause des grands nombres de *Pisidium casertanum* et *P. ferrugineum* qu'on y trouve. Des différences significatives de densité des mollusques dans deux lacs d'alcalinité intermédiaire indiquent que des facteurs autres que l'alcalinité peuvent influencer la distribution des mollusques. On a trouvé la clam unionide *Elliptio complanata* dans deux lacs d'alcalinités moyennes de 0,41 $mg \cdot L^{-1}$.

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Our study was initiated in response to recent reports of declining mollusc populations in regions experiencing acidic precipitaiton (Weiderholm and Eriksson 1977; Okland 1980; Raddum 1980). Compilers of lake surveys have noted the absence or suspected loss of molluscs in many areas of the world and have suggested that the absence of certain species could be used as an indicator of low alkalinity (Almer et al. 1974; Dussart 1979; Okland 1979; Okland and Okland 1980). This hypothesis is usually supported by the argument that molluscs require large amounts of calcium carbonate to construct their shells (Boycott 1936; Macan 1950) and that under conditions of low alkalinity little carbonate is available in the environment. At very low alkalinity levels, previously formed shells can also become severely corroded (Singer 1981; Strayer et al. 1981; Okland and Kuiper 1982).

In addition to these studies, primarily European, there is some evidence to suggest that mollusc populations of low-alkalinity waters in Ontario differed from those of higher alkalinity waters in adjacent areas. McKillop and Harrison (1972) showed characteristic changes in mollusc community composition across an interface between Canadian Shield (softwater lakes) and limestone formations (hardwater lakes). Roff and Kwiatkowski (1977) surveyed the benthos of several low-pH lakes and noted that few molluscs were present in the most severely stressed lakes. A more detailed survey (Mackie and Flippance 1983a) showed that, in general, there were fewer species and lower numbers of molluscs in lowest alkalinity environments. However, many of these surveys employed few collections at each site and sites were usually visited only once during the year or only during one season.

We examined the mollusc populations in a small number of low-alkalinity lakes and sampled them at six different depths during three seasons. By taking many samples at each visit and returning many times throughout the year we hoped to gain a better understanding of molluscan occurrence in low-alkalinity lakes that are currently experiencing acidic precipitation (Harvey et al. 1981).

Study Area

The six study lakes, situated on the Precambrian Shield near Dorset, Ont. (Fig. 1), have been monitored by the Ontario Ministry of the Environment for a number of years. Despite obvious differences in size and depth among the lakes (Table 1) they are generally representative of the region. All are contained within granitic basins of Precambrian silicate rock, with low solubility and very limited acid-neutralizing capacity (Chapman 1975). Mixed coniferous and deciduous forests cover the watershed of each lake and except for Blue Chalk Lake, all have adjacent areas of wetlands. Shoreline cottage development varies from almost complete for Dickie Lake and Harp Lake to none for Plastic Lake.

Plastic Lake and Heeney Lake had the lowest average alkalinity and calcium concentrations, determined from sam-

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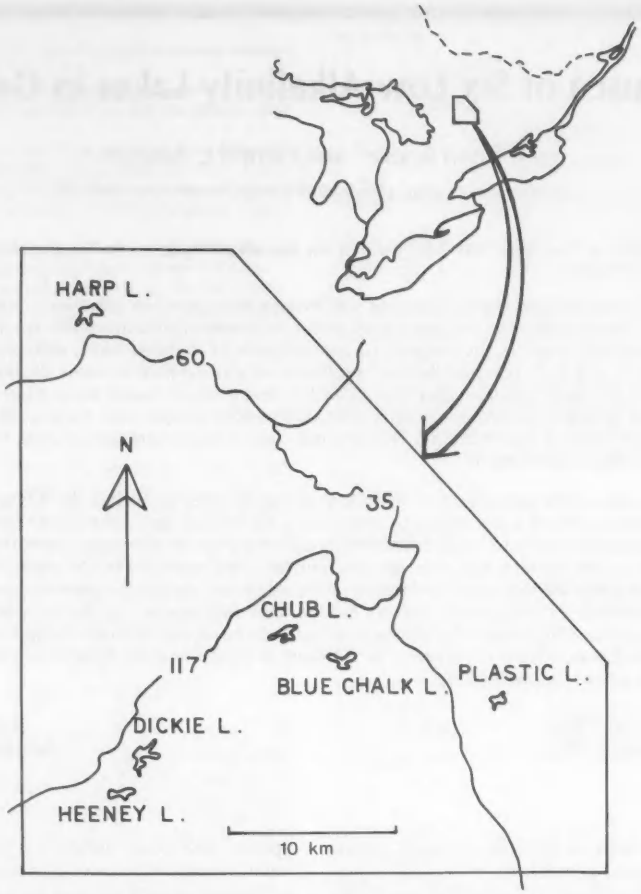

FIG. 1. General location of study area in southern Ontario and detailed location of lakes within the Haliburton region, in relation to Provincial Highways 35, 60, and 117.

ples taken (by the Ontario Ministry of the Environment) biweekly from the epilimnion between April and October (Table 1). Blue Chalk Lake and Harp Lake had the highest alkalinity and calcium concentrations whereas Dickie Lake and Chub Lake had intermediate levels. All six lakes had alkalinity and calcium levels at the low end of the range found in natural waters and could be termed softwater lakes (Wetzel 1977). Sulfate concentrations were not correlated with either alkalinity or calcium (Table 1). Further chemical and physical information concerning these lakes may be obtained from the Ontario Ministry of the Environment.

Materials and Methods

We sampled molluscs from the six study lakes approximately biweekly from early May to late October in 1980 and 1981. Ten transects were established on each lake (Fig. 2), and transect locations were chosen to ensure that all habitats (shorelines, bays, and depths) of each lake were sampled. Preliminary analysis of collections from the first sampling period indicated that a depth-stratified sampling scheme along the transects resulted in estimates of mollusc abundance that had a 23–30% error of the mean, a level of uncertainty that was deemed acceptable.

One Ekman grab sample was taken at each of 0.5-, 1-, and 3-m depths on each transect in 1980. Similarly, single samples were taken at 5-, 7-, and ≈11-m depths on each transect in 1981. There was a division of the sampling effort over 2 yr because it was physically impossible to carry out the entire program in 1 yr. However, comparing a small number of samples taken from the deeper waters of all lakes in 1980 with similar samples taken in 1981 indicated that there was no difference between the numbers of molluscs collected in the 2 yr (t-test, $P = 0.05$). We suggest that the deepwater populations did not change between the 2 yr and that use of 1981 deepwater samples with 1980 shallow-water samples gives a true representation of mollusc populations in the whole lake.

On one occasion a scuba diver checked the validity of the sampling procedure by taking replicate quadrat samples alongside those collected with the Ekman grab operated from a boat. There was no significant difference in the mean number of molluscs collected by these two methods when samples were taken from relatively soft substrates (ANOVA with Duncan's multiple range test at $P = 0.05$). When gravel and rock were

TABLE 1. Selected morphometric and chemical characteristics of the study lakes (data provided by the Ontario Ministry of the Environment). Values followed by the same letter are not significantly different ($P = 0.05$, $N = 10$). These values are averages of samples taken in the epilimnion, approximately biweekly during the growing season of the molluscs (April–October, 1980).

	Plastic	Heeney	Dickie	Chub	Blue Chalk	Harp
Surface area (ha)	32.2	21.7	93.2	32.2	49.4	66.9
Volume (10^4 m^3)	258.0	72.4	464.0	285.0	421.5	826.0
Maximum depth (m)	17.0	6.5	12.0	27.0	23.0	40.0
Mean depth (m)	8.0	3.3	5.0	8.9	8.5	12.4
Alkalinity (mg·L^{-1})	0.25 a	0.24 a	0.41 c	0.41 c	3.17 b	2.77 b
Calcium (mg·L^{-1})	2.06 a	2.08 a	2.48 c	2.53 c	2.99 b	3.15 b
Sulfate (mg·L^{-1})	6.81 a	7.43 b	7.38 b	8.04	7.05 a	8.52

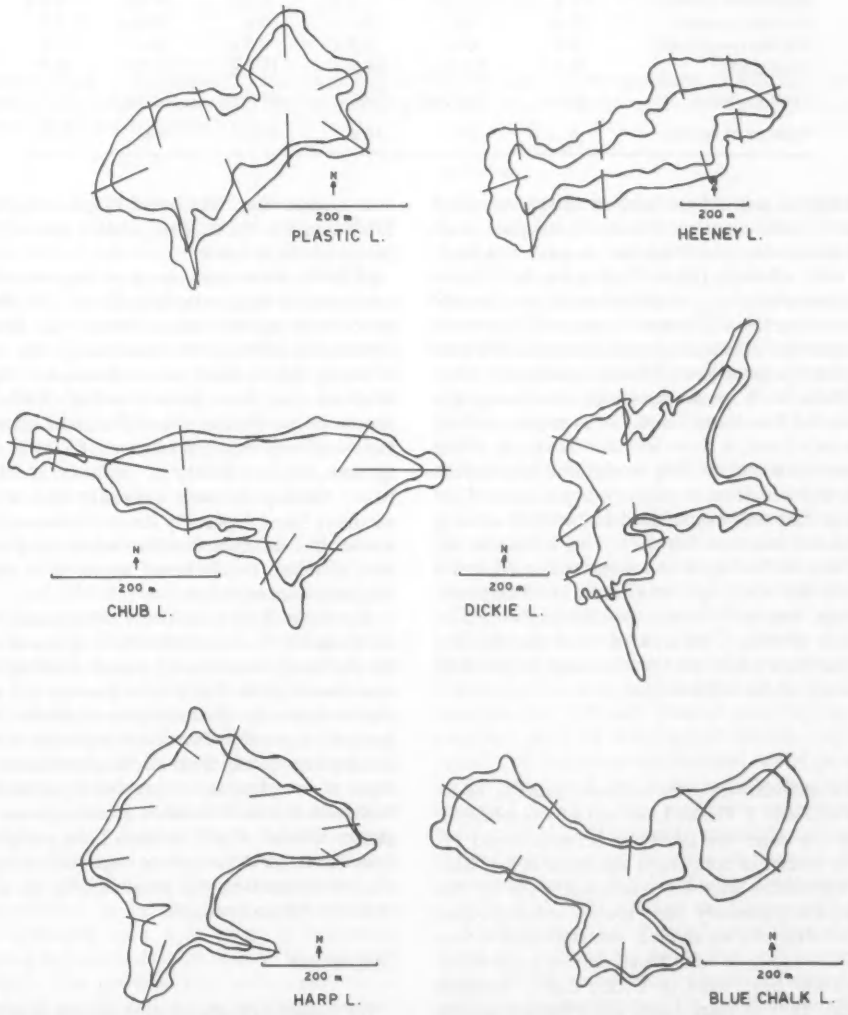

FIG. 2. Six study lakes with location of the 10 transects on each lake. The single contour shown is at 4 m, delimiting the shallow areas sampled in 1980 and the deep areas sampled in 1981 (see text).

TABLE 2. Mean number (per square metre) of each mollusc species found in the six study lakes. Densities are based only on the depth strata in which each species was actually found (see text). Values followed by the same letter are not significantly different ($P = 0.05$) compared within one row and across all columns ($N = 11$ (11 sampling dates)). Lakes are ranked left to right in successive pairs of increasing alkalinity.

	Plastic	Heeney	Dickie	Chub	Blue Chalk	Harp
Amnicola limosa	0.4	7.4 a	8.9 a	11.6 b	14.9 b	20.0
Gyraulus parvus	0.5 a	0.4 a	1.1 a	0.4 a	0.5 a	0.8 a
Helisoma anceps	1.9 a	1.6 a	2.4 a	0.5 b	1.7 a	0.8 b
Ferrissia parallela	0 0	2.5 a	0.6	6.1	3.7 a	1.6
Campeloma decisum	0 a	0 a	14.1	0 a	0 a	0 a
Total snails	2.8	11.4	27.1 a	18.7 a	20.8 a	22.8 a
Pisidium casertanum	21.5	37.1 a	30.6 a	9.9 b	8.4 b	11.8 b
P. ferrugineum	30.7	18.3 a	16.3 a	5.9	1.7 b	2.4 b
P. equilaterale	0	1.4 a	1.2 a	0.9 a	1.4 a	0.4
P. variable	0 b	2.1 a	2.2 a	1.0 b	0 b	1.4 b
P. adamsi	5.3	0 a	0 a	0 a	0 a	0 a
P. conventus	0 a	0 a	0 a	0 a	0 a	10.7
Musculium securis	0 a	1.2 a	1.8 a	0.6 a	0.5 a	0.7 a
Andonta grandis	0 a	0 a	0 a	0 a	0.9 a	1.1 a
Elliptio complanata	0 a	0 a	1.4 a	0.8 a	0 a	0 a
Total clams	57.5 a	61.1 a	52.5 a	19.0 b	12.9 b	38.5
Total molluscs	60.3	72.5 a	79.1 a	37.7 b	33.7 b	51.3
Number of species	6	9 a	11 a	10 a	9 a	11 a

encountered the Ekman grab did not function well and the diver collected 1.5 to 3 times more gastropods; pelecypods were rarely found on those substrates. Therefore, in lakes with large proportions of rock substrate (Blue Chalk Lake and Dickie Lake), gastropods may have been considerably underestimated.

In an attempt to correct for differences in area and depth of the study lakes, comparisons of mollusc populations (ANOVA with Duncan's multiple range test at $P = 0.05$) were made on the basis of the mean number of each species per square metre using only the depth strata in which each was found. For example, consider a species that is only found in water less than 5 m deep; taking the total lake population and dividing by the total lake surface area would indicate more dense populations in a small shallow lake than in a large deep one, even if the densities were actually the same in the water less than 5 m deep. This is because the "unoccupiable" area of the larger lake would act to dilute the densities there. In this study the "area" used to establish the compared densities was that of only the strata known to be occupied by each species. The area of each stratum was determined by planimetry from bathymetric maps supplied by the Ontario Ministry of the Environment.

Results

The occurrence and densities of the molluscs found in the six lakes are shown in Table 2. Plastic Lake had only 6 species of mollusc whereas the other five lakes had between 9 and 11. There were fewer species of both clams and snails in that lake, although these differences were not significant when the two groups were compared separately. Most species occurred across the entire range of alkalinity we studied, although often at very low densities. Three molluscs were found only in single lakes: *Campeloma decisum* Say, 1816 in Dickie Lake, *Pisidium conventus* Clessin, 1877 in Harp Lake, and *Pisidium adamsi* Stimpson, 1851 in Plastic Lake. Although there was no statistical difference in the numbers of unionid clams (*Anodonta grandis* Say, 1829 and *Elliptio complanata* Lightfoot, 1786) found in these lakes, neither species was found in the lowest alkalinity lakes.

Molluscs were more dense in lakes with lowest alkalinity concentration (approximately $72 \cdot m^{-2}$ in Heeney Lake compared with approximately $34 \cdot m^{-2}$ in Blue Chalk Lake). Differences between the densities in the two intermediate-alkalinity lakes, however, indicate that factors other than alkalinity may have been involved (Table 2). The greater density of two pisidiid clams (*Pisidium casertanum* Poli, 1795 and *Pisidium ferrugineum* Prime, 1852) was responsible for the apparent greater density of molluscs in the lower alkalinity lakes. Gastropods were generally less dense in the lower alkalinity lakes, especially *Amnicola limosa* (Say, 1817). Other species had different densities when compared between lakes but individual trends never appeared to be associated with alkalinity concentrations.

The depth distributions of *A. limosa* and *P. casertanum* are shown in Fig. 3. *Amnicola limosa* appeared to be restricted to the shallower waters in the lower alkalinity lakes whereas *P. casertanum* depth distribution patterns did not seem to vary consistently with alkalinity concentrations. Both species were generally more abundant in the shallower waters of each lake. Comparison of absolute depth distribution (e.g. maximum depth of occurrence) was precluded by the differences in the maximum depths of the lakes. Hence, species could be found at greater absolute depth in Harp Lake compared with Heeney Lake due to the much greater depth of the former lake. Only a relative comparison was possible (Fig. 3); rigorous statistical treatment was not possible.

Discussion

We found that molluscs were present in six Ontario lakes that currently have low alkalinity levels. Four of the six lakes had mean alkalinity concentrations of less than $0.5\,mg \cdot L^{-1}$ (as

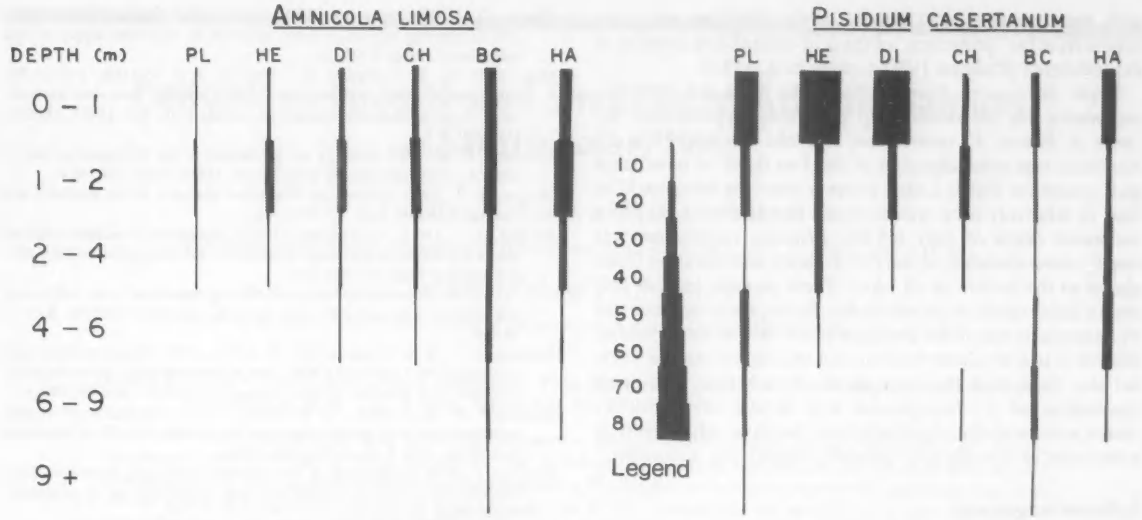

FIG. 3. Depth distribution of *A. limosa* and *P. casertanum*. Legend indicates width of bar representing various mean numbers per square metre. Lakes are ranked left to right in successive pairs of increasing alkalinity. PL, Plastic Lake; HE, Heeney Lake; DI, Dickie Lake; CH, Chub Lake; BC, Blue Chalk Lake; HA, Harp Lake.

calcium carbonate), but in only one lake were there fewer species than in the other lakes. Different combinations of species were present in each lake but no consistent relationship could be found between species occurrence and lake alkalinity.

However, unionid clams, either *A. grandis* or *E. complanata*, were found in all lakes except the two with lowest alkalinity (Table 2). *Elliptio* was found in isolated populations in both Dickie and Chub lakes at alkalinities well below (0.41 $mg \cdot L^{-1}$) the assumed lower threshold of alkalinity tolerance for these animals (Okland and Okland 1980; Singer 1981). Mackie and Flippance (1983a) found *E. complanata* in less alkaline waters (2–172 $mg \cdot L^{-1}$) than *A. grandis* (3.6–280 $mg \cdot L^{-1}$), and the presence of these two species in our six lakes is well below their previously recognized range.

In a survey of 53 aquatic habitats, Mackie and Flippance (1983a) also found molluscs in low-alkalinity waters. Other researchers have found molluscs under similar conditions but most assumed that the molluscs would die if alkalinity declined further (Grahn et al. 1974; Weiderholm and Eriksson 1977; Raddum 1980). We have shown here that molluscs are not necessarily absent from low-alkalinity waters in Ontario and that their use as indicators of this condition, on a simple presence–absence basis, would not be satisfactory. Indeed, we have shown that even "sensitive" unionid clams may be found when a rigorous sampling program is employed.

Molluscs were more numerous in three of the four lakes with alkalinity below 0.5 $mg \cdot L^{-1}$ compared with the other study lakes. This was the result of large numbers of two pisidiid clams in Plastic, Heeney, and Dickie lakes. One of these species, *P. casertanum*, is considered to be the most widely tolerant freshwater clam, appearing in a wide range of ecological settings and surviving in disturbed environments (Mackie et al. 1980; Clarke 1981). The pisidiids may even enjoy some ecological "advantage" as indicated here by their high densities in the lower alkalinity waters.

Some molluscs, however, cannot tolerate low alkalinity and hence are found in low numbers or are absent under such conditions (Mackie and Flippance 1983a). The hydrobiid snail, *A. limosa*, is a good example of this phenomenon. This species was most abundant in the higher alkalinity lakes and very rare in Plastic Lake. Considering both pisidiids and *Amnicola*, however, the differences between densities in the two intermediate-alkalinity lakes (Dickie Lake and Chub Lake) indicate that factors other than alkalinity may be operating or that some type of threshold of effect may occur at or around 0.4 $mg \cdot L^{-1}$. Further studies on a larger number of lakes are required to clarify this problem.

Mackie and Flippance (1983b) gave evidence to indicate that the relative success of *P. casertanum* may be due to high organic content and/or thick periostracum of the shell. They also showed that many pisidiids respond to low alkalinity by forming short heavy shells that presumably offer more protection against corrosion by hydrogen ions than do long thin shells.

There are at least two possible reasons why pisidiid clams could be more resistant than *A. limosa* to low alkalinity. Pisidiids are ovoviviporous and retain their young until they are released as miniature replicas of adults. Inside a shell of almost pure clacium carbonate (Mackie and Flippance 1983b) is an excellent place for development because any carbonate deficiencies of the natural environment can be avoided. *Amnicola*, on the other hand, lays eggs one at a time and these eggs are protected only by a thin permeable membrane, across which both calcium and carbonate requirements must be satisfied. Pisidiid embryos may, therefore, be protected from the external environment while they develop but *Amnicola* embryos may not (Collins et al. 1981; Okland and Kuiper 1982).

Another reason for the success of the pisidiids, relative to *Amnicola*, could be the habitat that each occupies. *Amnicola* is almost strictly epibenthic, living on the surface of the substrate and macrophytes (Berry 1943), but *Pisidium* spp. spend most of their time burrowing and only rarely come to the surface (Jonasson 1972). Thus, *Amnicola* is always in direct contact

with the (low-alkalinity) waters, while *Pisidium* spp. may benefit from the "protection" of the acid-neutralizing capacity of the sediments (Raddum 1980; Collins et al. 1981).

Depth differences among these lakes were not primarily responsible for the relationship previously hypothesized between *A. limosa*, *P. casertanum*, and lake alkalinity (Fig. 3). *Amnicola* was most abundant at the 1-m depth in most lakes and, except for Plastic Lake, where it was very rare, could be fond in relatively deep waters (recall that Heeney Lake has a maximum depth of only 6.5 m). *Pisidium casertanum* was usually most abundant at the 0.5-m depth and could be found almost to the bottom in all lakes. These patterns indicate that even if depth could be a confounding factor, the conclusion that *P. casertanum* was more abundant and *A. limosa* less abundant relative to low alkalinity was true not only in the shallow water but also to at least the mean depth of each lake. The depth distribution of *P. ferrugineum* was similar to that of *P. casertanum*, and other molluscs were found so infrequently in some lakes as to make even relative comparisons impossible.

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Patterns in Growth and Smolting of Atlantic Salmon (*Salmo salar*) Parr

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A simple model of growth and smolting of Atlantic salmon (*Salmo salar*) parr in Western Arm Brook (northern Newfoundland) can account for the distributions of ages and lengths of random samples of sea-run smolts collected over 10 yr. The parameters of the model determine growth rate, survival rate, and the length that a parr must attain to smolt at age 3 or 4. The parameters are specific to a year-class, not a calendar year, and vary between year-classes. To account for the observed patterns, it is necessary to assume that the criterion length for smolting is different for age 3 and age 4 parr. The variation between year-classes is as great for the criterion length for an age 3 smolt as for the expected length of an age 3 parr, and similarly for age 4. The best estimate of survival rate generally does not vary between year-classes.

La distribution des âges et des longueurs d'échantillons aléatoires de smolts d'avalaison de saumon atlantique (*Salmo salar*) prélevés dans le ruisseau Western Arm (nord de Terre-Neuve) sur une période de 10 ans peut s'expliquer par un simple modèle de croissance et smoltification des tacons. Grâce aux paramètres du modèle, on peut déterminer le taux de croissance, le taux de survie et la longueur que doit atteindre un tacon pour se transformer en smolt à l'âge 3 ou 4. Les paramètres sont spécifiques à la classe d'âge et non à l'année civile et varient d'une classe d'âge à l'autre. Pour expliquer ce qui a été observé, il faut supposer que la longueur critère de smoltification diffère chez les tacons d'âge 3 et d'âge 4. La variation entre classes d'âge est aussi grande pour la longueur critère de smolts d'âge 3 que pour celle anticipée de tacons d'âge 3, et il en est de même pour les tacons d'âge 4. La meilleure estimation du taux de survie ne varie généralement pas d'une classe d'âge à l'autre.

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An important part of salmon management is the prediction of the number of salmon that a spawning stock can produce in a generation. The prediction of numbers breaks naturally into three parts of the life cycle: the number of eggs hatching as a function of the spawning stock size, the number of smolts leaving a stream as a function of eggs hatching, and the number of returning adults as a function of smolt run. In principle, each of these functions might reach a maximum value at some finite value of its argument and decline for larger values; thus, there will be a maximum size of spawning stock. The prospects for density-dependent production are especially intriguing in the smolt production function. It is generally believed that salmon parr will not become smolts until they have reached a certain minimum length (Elson 1957) and also that they can migrate to the sea during only a small part of the year (Chadwick 1982). A small reduction in the growth rate of a parr might be enough to delay its smolting, and consequently subject it to stream mortality, for another year. The study of density-dependent smolt yield is therefore both managerially important and scientifically subtle.

Of course, not all the variability in parr growth is density dependent: environmental variability will also play an important part, and there is no way to study density effects independent of environmental effects. It may, however, be possible to do the reverse, i.e. study a stream in which parr density is so low that density effects are negligible, and hope that what we learn about environmental effects on parr from such a stream can be transferred to more heavily stocked streams.

Our objective here is to study density-independent patterns of growth and smolting of salmon (*Salmo salar*) parr and how they vary. The most obvious way to study the growth of salmon parr would be to collect random samples of parr in different years and look at growth increments. The age of a parr can be determined from the number of rings on its scales. Parr have a fairly short feeding season (when insects are abundant in the stream) so that, if we collect our sample outside this season, we can determine the integer number of feeding seasons each parr has had. The problem is in making the sample random. There are many different types of stream habitat, and even if we knew what fraction of effort should be devoted to each type, not all parr in a habitat will be equally vulnerable to capture.

We can, however, collect a random sample of smolts by setting up a counting fence that retains every smolt for counting and optional measurement. Moreover, data on smolts are more nearly related to our initial questions about productivity and environment; parr were only a step along the way. Using an

existing data set, we shall address two questions, one methodological (what can observations of smolts tell us about patterns of growth and smolting of parr?) and the other biological (how do these patterns vary?).

Methods

The Data

Western Arm Brook is a pristine, fourth-order stream on the northwest coast of Newfoundland (51°11′N, 56°46′W). The watershed is small (150 km^2) and because of its low relief and over 2000 ha of lakes, there are no large fluctuations in discharge. It has been possible to maintain a fish counting fence, with no washouts, since 1971. Each year the smolt migrations were completely counted, and biological attributes such as fork length, age, and sex were taken from random samples. Complete data are available for eight year-classes, providing the longest and most complete time series of information on wild Atlantic salmon migrations in Canada, if not the world (Chadwick 1981, 1982). The intensity of sampling differed between years but is known for each year (Table 1). Where necessary, analyses were corrected for sampling intensity differences.

Chadwick (1982) described some interesting features of these data. First, there was a highly significant relationship between an estimate of egg deposition (stock) and smolt production (recruits) ($r^2 = 0.94$, $P < 0.01$), with no evidence of a fall-off at higher densities. This indicates that Atlantic salmon in Western Arm Brook were below carrying capacity and that density-dependent effects were probably unimportant. There appeared to be little variation among year-classes in mean (fork) length of smolts compared with some other attributes measured. For example, smolts ranged in length from 121 to 354 mm within a year-class, yet mean length of year-classes ranged only from 171 to 174 mm. As a rule, smolt length increased with age, and variation between year-classes in mean length was largely due to variation in the proportion of smolts at different ages. By contrast, mean smolt age of year-classes was highly variable. Smolts ranged in age from 2 to 7 yr, although all but 5 of over 1300 sampled were ages 3, 4, or 5. The proportion of age 3 smolts ranged form 9 to 41%, the proportion of age 4 from 55 to 83%, and the proportion of age 5 from 5 to 26% (Table 2). Although older smolts were generally longer than younger ones, their number of growing seasons was proportionally greater still; thus, older smolts had lower average growth rates.

Mean smolt age was significantly predicted by air temperature in the first year of life: the mean smolt age of year-classes with cold first years was reduced ($r^2 = 0.85$, $P < 0.01$). Chadwick (1982) suggested that this was due to the reduced cost of routine metabolism in cold years. However, he did not find a relationship between air temperature and back-calculated growth rates to support this explanation. The highly significant relationship between smolt age and temperature again suggested that there was little confounding from density-dependent factors in Western Arm Brook.

The Model

To relate the above observations to our questions about salmon growth and smolting, we face an inverse, or system identification, problem in which we are given the end product, the smolts, and asked to identify the process that led to them. The standard approach to such a problem is to postulate a model of the process, with several undetermined parameters, which for any choice of parameter values makes predictions (in this case about numbers and sizes of smolts) that can be compared with observations. We adjust the parameter values until the agreement between model and observations is as good as possible and then decide whether this agreement is good enough.

We have constructed a probabilistic model of the life of a parr as it grows until smolting or dying. The growth rate and the criteria for smolting are random variables. The model thus predicts probability distributions for the age of a smolt and for the length of a smolt of a given age. These distributions can be compared with the observed numbers at age and at length for a given age. The model does not predict the total number of smolts: this depends on the spawning stock size, which we do not consider here.

We assume that variability occurs between year-classes rather than between years. That is, the parameters that determine growth and smolting are set in or before the year of emergence of the eggs from the stream bed. (We do not believe that this is the complete truth. However, the strong correlation in Western Arm Brook between mean smolt age and temperature in the year of emergence suggests that it is a sensible place to start.) A 0+ parr starts life with a length of 28 mm and a growth rate chosen at random from a Gaussian distribution whose mean (MUG) and standard deviation (SIGG) are parameters of the model. It maintains this growth rate (millimetres per year, not percent per year) throughout its river life. This is consistent with Chadwick (1981, table 17), who used annular

TABLE 1. Total number of smolts counted during each calendar year and the yearly sampling intensity conversion factor for scaling numbers of sampled smolts for comparisons among years.

Calendar year	Number of smolts	Sampling intensity (% taken)
1971	5 734	0.96
1972	11 906	1.94
1973	8 484	1.01
1974	12 055	1.66
1975	9 733	2.41
1976	6 359	1.97
1977	9 640	1.78
1978	13 071	1.22
1979	9 400	1.95
1980	15 675	1.61
1981	13 981	1.32

TABLE 2. Distribution of all sampled smolts by age for the year-classes. These counts are not corrected for the sampling intensity differences among calendar years (Table 1).

	Age				
Year-class	2	3	4	5	6
1968	0	10	181	6	1
1969	0	27	67	43	0
1970	0	13	124	8	1
1971	0	24	141	16	0
1972	0	83	88	3	0
1973	2	19	72	12	0
1974	0	97	89	12	0
1975	0	59	126	9	1

radii of scales of smolts to back-calculate growth rates in each year of river life. He found differences in growth rate due to the age of smolting but no additional differences due to the year of life. The parr also starts life with a criterion length, the minimum length that it must attain before becoming a smolt, chosen at random from another Gaussian distribution, whose parameters (MUL and SIGL) are again parameters of the model. Parr mortality in the fourth and fifth river years is another parameter (SURV) and is assumed to be independent of age and growth rate. Because we are using only smolt data, and only relative numbers at different ages and lengths, we cannot determine the mortality of parr too small to smolt. The general sequence of events in the life history of a parr (and the exact sequence in the model) is grow, die (if it is going to), become a smolt (if it is going to), increment age, and repeat cycle. Because of this order, the actual length of a smolt may be much greater than its criterion length, if it just missed its criterion smolt length in the previous year.

A special case of the model, with SURV = 1 and SIGL = 0 (all parr survive to smolt, and all have the same criterion length) is illustrated in Fig. 1. It shows the distribution of growth rates and picks out the rate $a = (\text{MUL} - 28)/3$ that divides age 3 and 4 smolts and the rate $b = (\text{MUL} - 28)/4$ that divides age 4 and 5 smolts. Numbers at age are proportional to the areas under the appropriate parts of the curve. Each part separately, with suitably scaled axes, depicts the probability density function for the length of that age of smolt. The figure allows us to see the effects of changing different parameters. Increasing MUG shifts the curve to the right while keeping the dividing lines fixed. This will produce more age 3 smolts, and fewer age 5 smolts, and will increase the mean length of each age-class slightly. Decreasing MUL leaves the curve fixed but moves the dividing lines to the left. This will again produce more age 3 and fewer age 5 smolts but will markedly decrease the mean length of each age-class.

Once the five parameters are chosen, the model determines the probability distributions of numbers of smolts at each age and at lengths within an age. To compute these distributions, we choose a large number (2000) of random samples of growth rates and criterion lengths and grow smolts according to the recipe. No more than 200 smolts were sampled from any year-class; thus, discrepancies between model and observations can safely be ascribed either to noise in the observations or to a wrong model. We next describe the statistical tests that enable us to decide between these possibilities.

Statistical Tests

Accepting the model as a representation of a given year-class meant finding a set of parameters for the model variables so that the model predicted the correct ages and sizes of smolts. The distributions of ages and sizes are non-Gaussian; therefore, we used four nonparametric goodness-of-fit tests: one for the fit of predicted to observed numbers of smolts at ages 3, 4, and 5 and three for the fits of predicted sizes of age 3, 4, and 5 smolts to observed sizes. We used a *G*-test (Sokal and Rohlf 1981) to assess the fit of numbers at age (adjusting for differences in sampling intensity among years, and matching total smolt numbers). We used a Kolmogorov–Smirnov test (K–S test; Sokal and Rohlf 1981; exact probabilities taken from Birnbaum 1952) to assess the fit of predicted to observed size distributions. There is some question about whether we are testing an intrinsic or extrinsic hypothesis, i.e. whether the parameters of the model

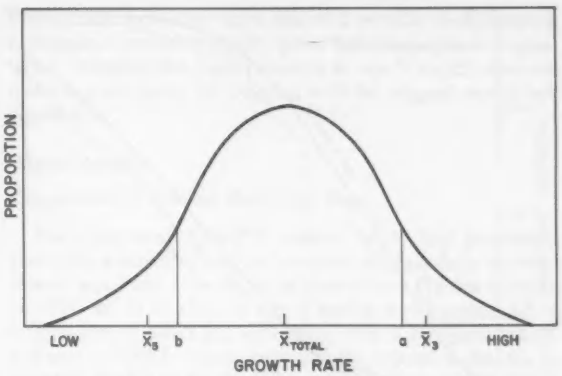

FIG. 1. Distribution of growth rates of the potential smolt population. The "leading" edge of the distribution (to the right of *a*) are parr with growth rates high enough to smolt at age 3. Note that because of the shape of that portion of the distribution the mean size of age 3 smolts is expected to be close to the smallest allowable smolt. The "trailing" edge of the distribution (to the left of *b*) are parr growing so slowly they will smolt at age 5. Here, the mean size is expected to be much higher than the size of the smallest smolt.

are being estimated from the data or are supplied by some theory from outside. Clearly, the overall model is intrinsic, but when we are testing one of the distributions, we must remember that the parameters must also be appropriate for three other distributions. Perhaps the hypothesis is one quarter intrinsic and three quarters extrinsic. Our approach has been to treat all the K–S tests as if they were testing extrinsic hypotheses. This has the effect of making rejection more difficult: the tolerable deviations are larger. We are going to reject the model for a large number of year-classes; thus, our choice is the more conservative.

Our criterion for accepting a set of parameters is that the predictions be accepted by all four tests with $\alpha \geq 0.05$. If there is an acceptable set of parameters, then there are many such sets, and our criterion compels us to accept all of them as possible representations of the truth. For some purposes it is useful to distinguish a single "best" set of parameters, which we take to be the set that maximizes the combined probability, using Fisher's method (Sokal and Rohlf 1981). This determination cannot be taken too literally because of the wide variation in power of the different tests being combined: hence, the quotation marks. For the same reason, we did not use Fisher's method in judging acceptability: we were hesitant to redeem a fit proved poor by 100 observations, using another fit accepted as good on the basis of 6 observations.

Results

Comparison of Model and Data

The model is able to represent fewer than half of the year-classes studied. The range of behavior of the model is best illustrated by considering two extreme year-classes: 1975 and 1974. For 1975, we were able to find a set of parameters that reproduced the observations extremely well (Fig. 2A; statistics of fit in Table 3). For 1974, the best fit we were able to find was poor (Fig. 2B; probabilities of doing that badly by chance are 0.001 for age 3 lengths and 0.003 for age 4 lengths).

Parameters were found that provided acceptable fits to the 1971, 1972, and 1975 year-classes. For all three year-classes,

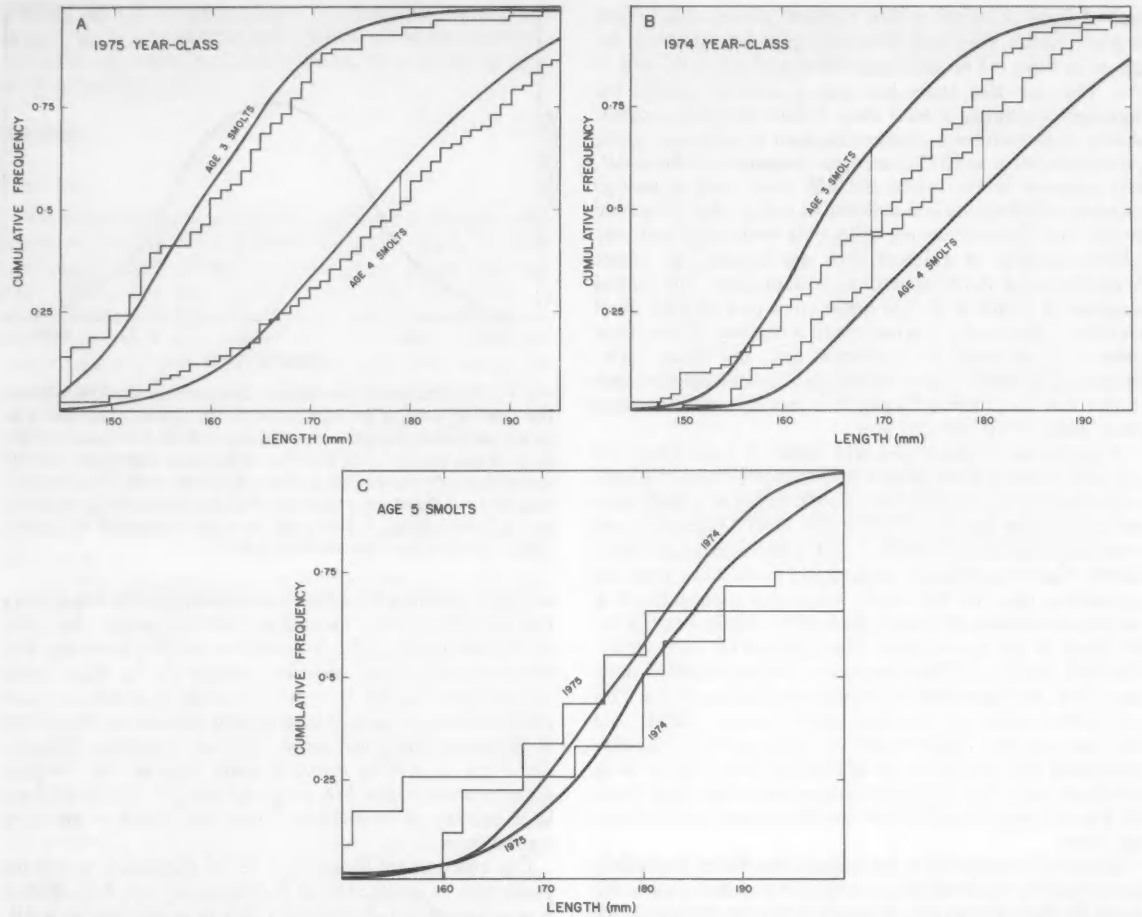

FIG. 2. Goodness-of-fit of predicted to observed (stepped line) cumulative frequency distributions of proportion at length for 1975 year-class, ages 3 and 4 (A), 1974 year-class, ages 3 and 4 (B), and 5-yr-olds, 1975 and 1974 (C). Vertical lines illustrate D_{max} for observed–predicted cumulative frequency distributions for each year-class.

fits to numbers at age were good (all $P > 0.25$), and for 1975 the fits to the size distributions of 3-, 4-, and 4-yr-olds were also good. For the 1971 year-class the best set of parameters provided only marginal fits to ages 3 ($P = 0.057$) and 5 ($P = 0.071$). For the 1972 year-class, the best set of parameters provided marginal fits to lengths of ages 3 ($P = 0.112$) and 4 ($P = 0.075$) (Table 3).

The marginal fits to attributes of the 1971 and 1972 year-classes represent compromises among different criteria and cannot be noticeably improved overall. For example, for the 1972 year-class the maximum deviation between observed and predicted length distributions (D_{max}) for age 3 smolts occurs at 170 mm, above the median of 167 mm, whereas D_{max} for age 4 occurs at 163 mm, below the median of 180 mm (Fig. 3A). The marginal D_{max} value for age 3 could be decreased by either decreasing the slope of the cumulative frequency distribution (i.e. by increasing SIGG or MUL; Fig. 3B) or by shifting the entire distribution to the right (increasing MUG; Fig. 3B). All three of these parameter changes increase D_{max} for age 4 (Fig. 3C). Because of the larger numbers of age 4 smolts than age 3, the distribution of age 4 sizes becomes statistically unacceptable faster than the fit to age 3 sizes is improved. Similar constraints prevent improvements to the fits to sizes of the 1971 year-class.

We found no parameters producing predictions that met our criterion of acceptance for any other year-class. Partial successes such as fitting all size distributions but not numbers were possible, but uninterpretable. More interestingly, the failure to fit the other year-classes was not due to lack of imagination in parameter searches: in the Appendix we give an argument that no parameters exist that could provide acceptable fits of model predictions to data from the remaining year-classes. Briefly, we find that in attempting to fit these year-classes, the model would produce too few age 4 smolts compared with age 3 and 5 smolts, too few long age 3 smolts, and too many long age 4 smolts. The same pattern was noticeable, although not significant, in the 1971 and 1972 year-classes. We argue that it is impossible to find a parameter set that will remedy all three of these deficiencies at once.

TABLE 3. Parameters providing "best" fits to 1971, 1972, and 1975 year-classes under the basic model (equivalently with OFFSET = 0) and statistics of fit to sizes and numbers for those year-classes (G with Williams' correction).

	1971	1972	1975
Parameters			
MUG	35.06	38.47	37.00
SIGG	5.23	5.69	5.30
MUL	157	158	156
SIGL	5	3	7
SURV	0.35	0.35	0.40
Statistics of fit			
Age 3			
D_{max}	0.269	0.130	0.093
P	0.057	0.112	0.652
Age 4			
D_{max}	0.094	0.135	0.073
P	0.167	0.075	0.523
Age 5			
D_{max}	0.312	0.325	0.324
P	0.071	0.787	0.302
Numbers			
Age 3			
Observed	24	83	59
Model	26	79	50
Age 4			
Observed	141	88	126
Model	139	89	133
Age 5			
Observed	16	3	9
Model	16	6	11
G	0.090	1.29	1.65
P	>0.95	>0.50	>0.50

Intermezzo

A Reason and a Remedy for Discrepancies

We believe that the model fails for five year-classes because smolting depends on age as well as size. The model we started with was basically constructed before any of the tests were done: it is a good a priori model. At this point we believe that a better model would incorporate a tendency for older parr to smolt at a lower criterion length, which would increase the number of age 4 smolts by allowing more short ones. (It would not help simply to reduce the growth rate in the fourth year because, although this would reduce the number of long age 4 smolts, it would also reduce their total number (fewer would attain the criterion length) and there are already too few.) The modification of the model has been suggested to us by a long consideration of the data set, and therefore it is not strictly legitimate to use the data to test this new model. The fact that it is the only solution we can think of may indicate only lack of imagination. With these reservations, that is nonetheless what we have done: introduced a new parameter, OFFSET, the amount by which the criterion length decreases with each successive year of parr age. At the same time, we altered our criterion for accepting the model. It became clear that age 5 smolts are freaks. In the 1968 year-class, for example, 4/6 of the age 5 smolts observed were $\leq$152 mm, while in the 1973 year-class, 10/12 were $\geq$191 mm. Therefore, we stopped trying to fit their lengths (although we still require a fit to numbers). There are various rationalizations for this: age 5 smolts are represented by very few individuals, which come from the weird tail of a possibly well-behaved distribution, and their scarcity gives them no pragmatic importance. Dropping the requirement to fit age 5 lengths does not make any additional fits possible with the original model (see Appendix).

More Results

Comparison of Revised Model and Data

The addition of OFFSET enabled us to find parameters providing acceptable predicted numbers at age and size distributions of age 3 and 4 smolts for all year-classes (Tables 4 and 5). In 1974 the fit to sizes of age 4 smolts was marginal (P = 0.055), but all other fits were better than 0.10. Furthermore, nonzero OFFSET values substantially improved the fits to length distributions for the 1971 and 1972 year-classes, where previously (OFFSET = 0), fits were marginal. For 1969, the only year-class with more than 20 age 5 smolts, we could also fit their length distribution well. Clearly, lowering the criterion smolt size from year 3 to year 4 of parr life enables model predictions to coincide closely with field data.

With one exception, all parameter values are intuitively reasonable. Survival for the 1969 year-class must be extraordinarily high (>0.80, and "best" at 0.95 or even higher) before acceptable fits to numbers at age are possible. Such rates of survival are unrealistic, but the data from the 1969 year-class are anomalous. No other year-class has as high a proportion of age 5 smolts (Table 2). Model structure does not allow us to investigate whether growth or smolting was aberrant for this year-class, although growth or smolting irregularities are at least as likely as survival rates between age 3 and age 5 of more than 90%. The 1969 year-class does illustrate that whatever general patterns are found, exceptions will occur. Aside from the anomalous survival for the 1969 year-class, however, the model with six parameters, mean and standard deviation of growth rate, mean and standard deviation of criterion smolt length, change in criterion smolt length between year 3 and year 4, and survival, is adequate to reconstruct the observed numbers and sizes of smolts for the Western Arm Brook salmon population.

Exploring the Space of Acceptable Parameters

We have not developed a proof that the neighborhood around any acceptable set of parameters we found is the unique neighborhood of parameters for meeting our multiple criterion of acceptance. Three lines of argument point in that direction, however. The first argument addresses the determination of initial parameter values for the modeling runs. This method is of special interest because it is a general approach to delineating limits for means and variances of underlying growth rates from any set of age-structured data on size.

First, we directly limited potential values for MUG and SIGG by calculating the simultaneous 95% confidence intervals (Kendall and Stewart 1969) of the mean and variance of growth rates of a year-class. These growth rates were for a "virtual" population of parr, hypothesizing a mortality rate, and converting observed lengths into growth rates as (length − 28)/age. Then, requiring good K–S fits to size distributions requires fitting the tails of the distribution of growth rates as well. Therefore, additional limits on the mean and standard deviation of growth rates could be set by the mean and 95% confidence interval of the mean for sizes, and therefore for growth rates, of

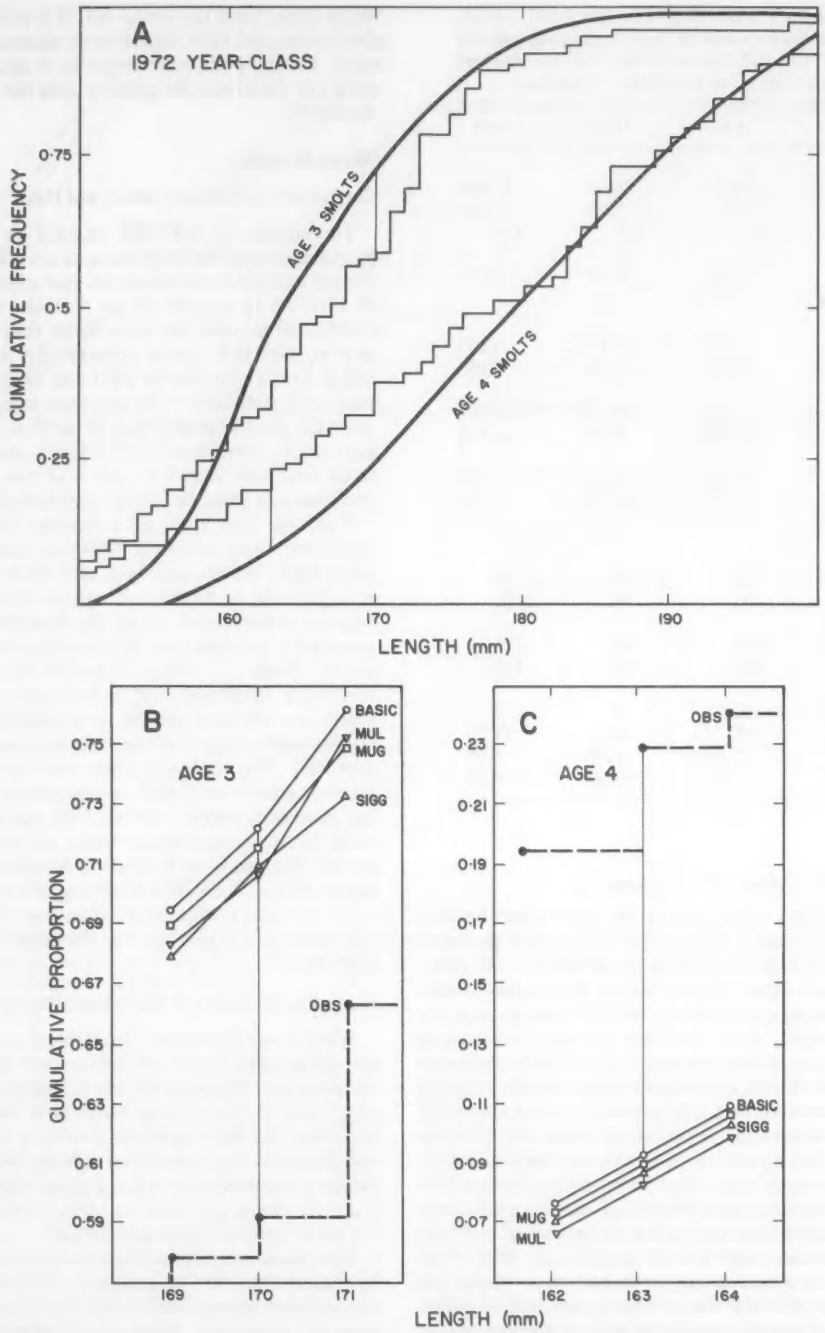

FIG. 3. Cumulative frequency distribution of observed (stepped line) and best compromise predicted (smooth line) proportions at length for 3- and 4-yr-olds of the 1972 year-class (A). There were too few 5-yr-olds to investigate fits with precision. Each parameter change that (B) decreases D_{max} (vertical line) for 3-yr-olds (increase MUG, increase SIGG, increase MUL) increases D_{max} for 4-yr-olds (C).

TABLE 4. Predicted numbers at age and statistics of fit of predicted sizes from revised model (with OFFSET) compared with observed population attributes. Model parameters for each year-class are listed in Table 5.

Year-class	Statistic	Age 3	Age 4	Age 5	G-test value
1968	Number	14.9	172.0	8.0	
	D_{max}	0.307	0.037	0.590	2.755
	P	0.270	0.998	0.032	0.330
1969	Number	32.0	63.3	41.7	
	D_{max}	0.152	0.101	0.172	1.116
	P	0.518	0.473	0.148	0.592
1970	Number	14.4	116.7	13.9	
	D_{max}	0.253	0.107	0.351	4.123
	P	0.344	0.119	0.247	0.158
1971	Number	20.7	143.7	16.6	
	D_{max}	0.213	0.056	0.312	0.548
	P	0.201	0.821	0.071	0.785
1972	Number	90.4	78.3	5.3	
	D_{max}	0.085	0.079	0.471	3.174
	P	0.559	0.614	0.496	0.278
1973	Number	15.6	80.2	7.2	
	D_{max}	0.263	0.139	0.824	3.767
	P	0.780	0.112	0.000	0.204
1974	Number	89.3	97.3	11.3	
	D_{max}	0.116	0.144	0.612	1.524
	P	0.094	0.055	0.000	0.483
1975	Number	65.6	118.8	9.7	
	D_{max}	0.079	0.086	0.300	1.379
	P	0.818	0.313	0.357	0.506

TABLE 5. Parameters providing "best" fits to each year-class for the model with nonzero OFFSET values.

Year-class	MUG	SIGG	MUL	OFFSET	SIGL	SURV
1968	35.6	5.91	164	16	5.0	0.40
1969	35.3	8.22	162	12	11.1	0.95
1970	36.2	5.50	163	12	7.0	0.40
1971	35.0	5.75	160	6	5.0	0.40
1972	38.2	7.00	158	8	6.0	0.40
1973	35.5	7.00	167	18	6.5	0.45
1974	37.0	7.29	160	15	6.0	0.40
1975	37.6	5.48	154	−1	8.0	0.40

3-, 4-, and 5-yr-old smolts. For the hypothesized mortality rate the proportion of the parr population with growth rates greater than or equal to the mean for each age group could be directly calculated and plotted (Fig. 4). The slopes of the lines connecting the end points of the confidence intervals limit the SIGG parameter (lines a and a' are upper limits, lines b and b' are lower limits). The points where the other connecting lines intersect ordinate position 0.50 limit possible values of the mean growth rate (lines c and c' are upper limits, lines d and d' are lower limits). This method of calculating parameter envelopes also can detect cases where underlying assumptions of normality of growth rate distributions are not valid, because the limits estimated from different portions of the data will not overlap and, hence, no single Gaussian distribution could have produced all of the observed size distributions. The envelope

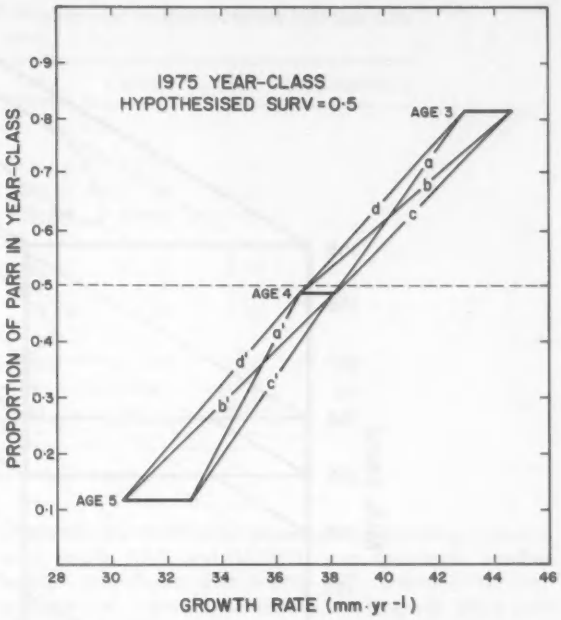

FIG. 4. Method for delimiting envelopes of initial parameter values for search for parameters providing fits to year-classes. Growth rates were calculated for all sampled smolts as (fork length − 28)/age. The 95% confidence intervals were calculated as mean length ± SE·$t_{0.05(df)}$ or using jackknife procedures if n at age < 12. Proportions were calculated with hypothesized survivorship of 0.50, taken from Chadwick (1982). Slopes of lines a, a', b, and b' limit candidate values of SIGG; intercepts of lines c, c', d, and d' with median (broken line) limit candidate values of MUG.

method of limiting MUG and SIGG was affected by errors in hypothesized mortality rates and deviations of growth distributions from normality, so the limits could only serve as guidelines. Nonetheless, the limits provided efficient starting points for sampling parameter space, and it is of interest to compare these points among year-classes even if other acceptable regions of parameter space might exist.

The second argument for the uniqueness of acceptable neighborhoods of parameter values is also empirical. For the 1975 year-class we delineated the entire acceptable parameter space around our "best" values. Although the space was elongated rather than spherical, it was convex. Values outside that delineated space were implausible for growth and smolting parameters that would fit observed values. Acceptable parameter spaces for other year-classes were not so fully explored but appeared similarly regular. The third line of evidence is the argument in the Appendix, which determines inequalities that all acceptable parameter sets must satisfy.

Because of the good fits to all three size distributions and to numbers at age, it was possible to explore the ranges of acceptable values for the growth and smolting parameters of the 1975 year-class. Although we will discuss parameters providing "best" fits, "best" will occur within quotes. There is a range of other parameter values we cannot reject as impossible. Nor, due to differences in precision in delimiting size distributions of different age groups within a year-class, can we argue strongly that our "best" parameters are unequivocally the best.

The joint acceptable ranges of growth and smolting para-

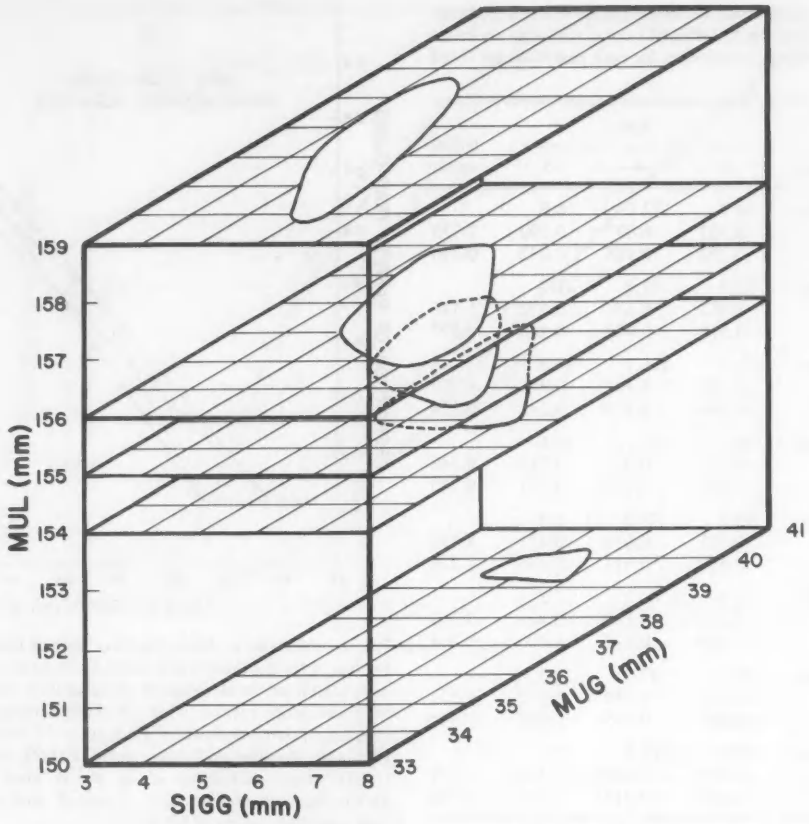

FIG. 5. Sections through the acceptable parameter space of MUG, SIGG, and MUL for the 1975 year-class. Parameters inside the space provide predicted size distributions of 3-, 4-, and 5-yr-olds and numbers at age that all match observed distributions or numbers with $P \geq 0.05$. SIGL was kept at 7.0 and OFFSET at 0.0 for all runs, but SURV varied from 0.10 (MUL = 159) to 0.95 (MUL = 150).

meters were determined by taking each parameter to its acceptable limit (the most extreme value that would not lead to rejection) and then altering the other growth and smolting parameters from their "best" values to see how much further each parameter could be pushed. Except when its limits were being explored, OFFSET was kept at 0 (very close its "best" value of −1.0) to simplify interpretation of the other parameters. Survival rates were altered as needed to keep the numbers at age within *G*-test limits. Parameter limits were almost always set by size distribution constraints rather than by the constraints of proper numbers at age. These extreme limits show the large parameter variation that is possible, even with data as comprehensive as those from Western Arm Brook.

The space delimiting acceptable values for MUG, SIGG, and MUL is an intersection of three ellipsoids (one for each of the statistical tests we are applying). The long axis reflects a negative association between MUG and SIGG within the year-class (Fig. 5). MUG and SIGG values chosen from the upper (MUL = 159) or lower (MUL = 150) extremes of the acceptable parameter space required respectively very low and very high values of SURV to fit numbers at age. SIGL had its widest range of acceptable values near the center of the acceptable parameter space. The ranges of acceptable values for SIGG and SIGL were smaller at all peripheral areas of the space (Fig. 5 and 6). The limiting acceptable coordinates for high MUG at the lowest possible MUL exceed their corresponding limits at the "best" value of MUL (156 when OFFSET = 0). Likewise, the highest possible value of MUL occurs with a lower MUG value than is acceptable at "best" MUL values. To fit numbers at age, slow growth rates required low survivals whereas fast growth rates required high survivals. The range of acceptable values for OFFSET was largest around the "best" values of the other parameters, but we did not explore fully its interactions with the other parameters.

Variability among Year-Classes

Having answered the first of our major questions, "what can smolt data tell us about growth and smolting patterns?", we turn to the second, "how do these patterns vary?" Different parameters cover very different scales of values, making comparisons of their ranges very difficult. Growth rates varied relatively little among year-classes (Table 5). It was sometimes true that values of MUG providing "best" fits to one year-class were not acceptable for other year-classes. For example, the growth rate of 38.25 mm·yr^{-1} that was "best" for the 1972 year-class was unacceptable for the 1968 year-class. More noteworthy, however, for the 1975 year-class, where the basic model predictions fit the data well, the range of acceptable

TABLE 6. Variation among year-classes in the "best" parameters and variation within the 1975 year-class in acceptable ($P > 0.05$ for all four tests) parameter values.

	MUG	SIGG	MUL	OFFSET	SIGL	MUL–OFFSET
Within year-class						
Total variation[a]						
Low	33.85	4.15	149.15	−7.10	5.75	
High	40.30	7.35	160.25	7.75	15.25	
Range	6.45	3.20	11.10	14.65	9.50	
Constrained variation[b]						
Low	35.90	4.25	149.25	−7.10	5.75	
High	39.05	6.75	157.10	7.75	15.25	
Range	3.15	2.50	7.85	14.65	9.50	
Among year-classes						
Low	35.06	5.48	154	−1	5.00	145
High	38.25	8.22	167	18	11.20	156
Range	3.19	2.75	13	19	6.12	11

[a]All parameters allowed to vary during search for fits.
[b]All parameters except the one under study are fixed at "best" values.

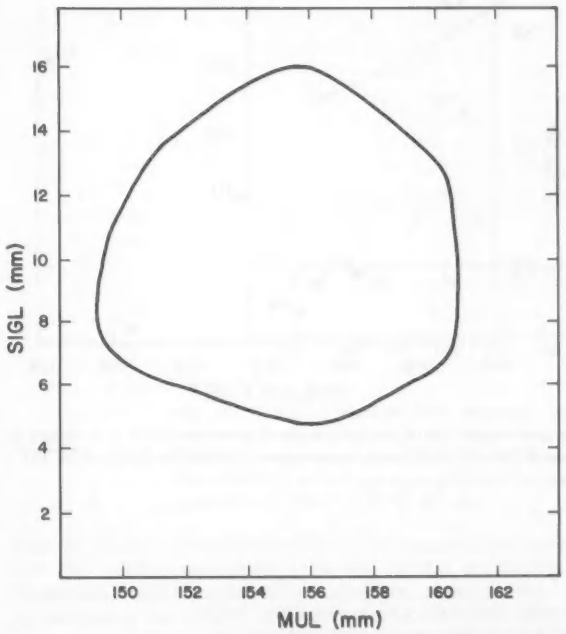

FIG. 6. Range of acceptable values of SIGL for various possible values of MUL. MUG and SURV took best values for the respective value of MUL as illustrated in Fig. 4.

values for MUG was twice as large as the range of "best" values among year-classes. In fact, even if all other parameters are kept constant at their "best" values when OFFSET = 0, the range of acceptable MUG values for the 1975 year-class is nearly the same as the between year-class range of "best" MUG values (Table 6).

It is enlightening to compare the acceptable variation in parameters within the 1975 year-class with the variation between year-classes. The between year-class variations in both standard deviations (SIGG and SIGL) were small and differed little from the ranges of acceptable values around the "best" parameters for 1975. The parameters determining criterion smolt length, MUL and OFFSET, were both more variable between year-classes than within 1975, however. We can conclude that, between year-classes, the length at which parr will smolt, both at age 3 and at age 4, is the most variable feature of those we considered. With the exception of the aberrant 1969 year-class, there is marked consistency in "best" values for SURV among year-classes (Table 5).

With the collection of points in parameter space given to us by the data, one for each year-class, we investigated whether any year-class characteristic is statistically correlated with any single parameter. We used Kendall's nonparametric correlation coefficient τ (Sokal and Rohlf 1981). Although MUL and OFFSET are logically correlated, neither parameter, nor their difference, is correlated with growth rate across year-classes (Fig. 7; $\tau_{MUL \times MUG} = -0.206$, $\tau_{(MUL-OFFSET) \times MUG} = 0.074$, both $P > 0.50$). Furthermore, MUL and OFFSET do not interact to produce smolts of a constant size for a calendar year (i.e. age 4 smolts of year-class i and age 3 smolts of year-class $i+1$ do not end up particularly similar in size; $\tau = -0.044$, $P > 0.50$; Table 7). Rather, the median size of age 4 smolts is quite similar among year-classes and is correlated with MUG (i.e. with the median size of age 4 parr) ($\tau = 0.655$, $P < 0.05$) but not with the criterion length of age 4 smolts (i.e. (MUL − OFFSET); $\tau = -0.056$; $P > 0.50$). Median sizes of age 3 smolts were much more different among year-classes and were determined by the criterion smolt length ($\tau = 0.674$, $P < 0.05$) rather than growth rate (MUL × MUG above). Hence, both growth rate and smolt length affect salmon smolting. Usually, age 4 smolts make up the majority of the production of a year-class. Their average size varies little among year-classes and is determined predominantly by growth rate. The lower size limit for them is more variable and set by MUL and OFFSET. How many parr become smolt as 3-yr olds and avoid an extra winter's mortality appeared legislated by a highly variable criterion length. This large variation in criterion length was quite unexpected. Although we have no biological explanation for the variation, it warrants thorough investigation.

Yield as a Function of the Various Parameters

To relate the insights into parr growth and smolting obtained

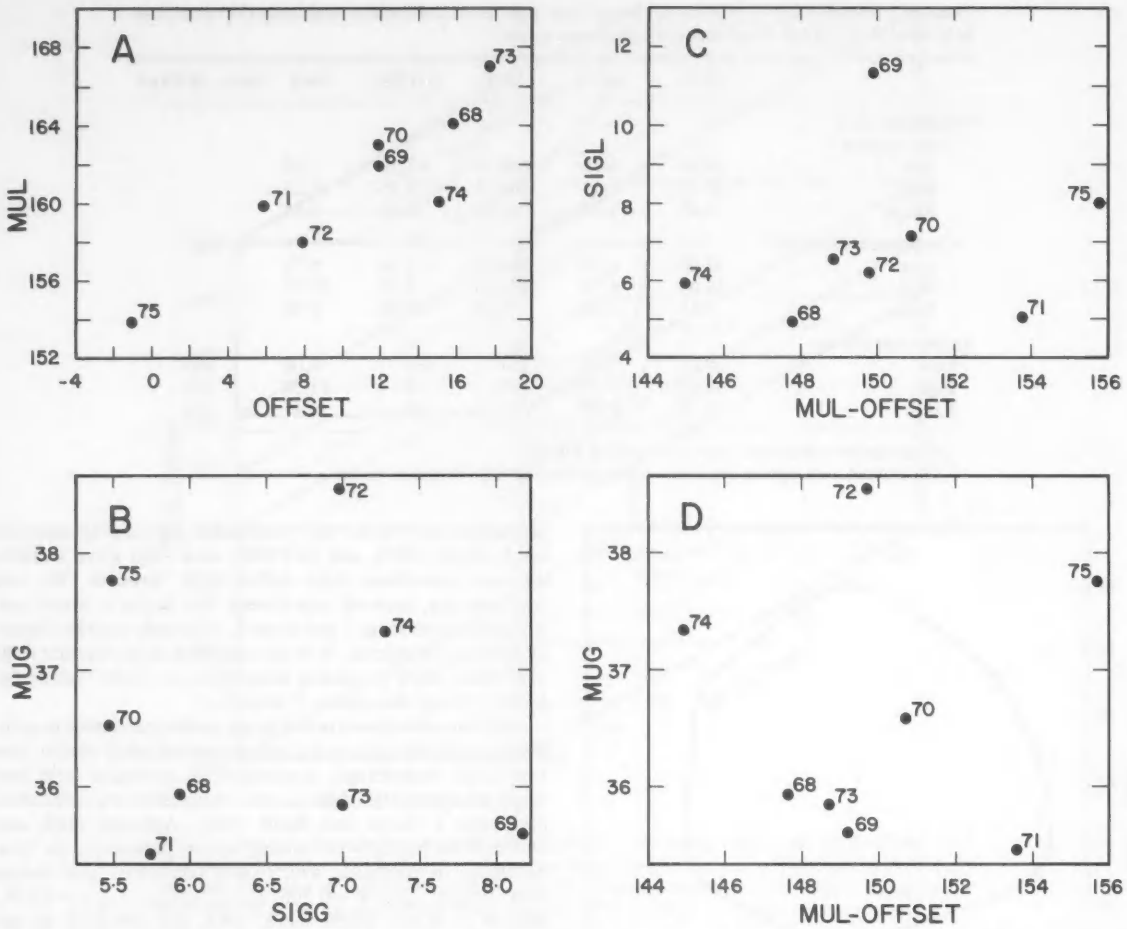

FIG. 7. Relationships among "best" values of model parameters across year-classes. (A) Criterion length of 3-yr-olds (MUL) vs change in criterion length (OFFSET); (B) Mean (MUG) and variation (SIGG) in growth rate; (C) SIGL and criterion size of 4-yr-olds (MUL–OFFSET); (D) MUG and criterion size of 4-yr-olds.

TABLE 7. Criterion length for age 3 and 4 smolts predicted by the model with "best" parameters for MUL and OFFSET and the median smolt length (mm) for smolts predicted for each age and year-class.

Calendar year	Age 3			Age 4		
	MUL	Median	Year-class	MUL–OFFSET	Median	Year-class
1971	164	169	1968			
1972	162	167	1969	148	174	1968
1973	163	164	1970	150	175	1969
1974	160	164	1971	151	175	1970
1975	158	166	1972	154	174	1971
1976	167	172	1973	150	178	1972
1977	160	167	1974	149	176	1973
1978	156	159	1975	145	175	1974
1979				155	177	1975

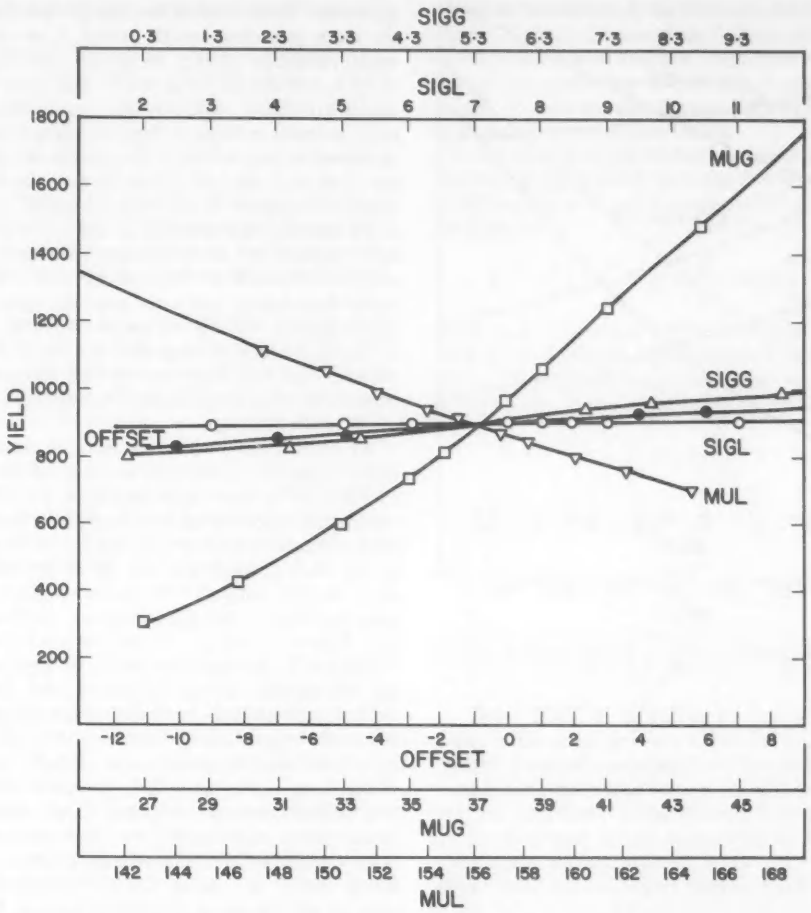

FIG. 8. Predicted yield of 1975 year-class (number of smolts produced from 2000 0^+ parr) as a function of mean growth rate (MUG), variation in growth rate (SIGG), criterion length of 3-yr-olds (MUL), change in criterion length (OFFSET), and variation in criterion length (SIGL). For determination of each yield, all parameters except the one under study were held at "best" values for the 1975 year-class. SURV = 0.40 for all runs.

from the model to management of the stock, one must determine how the various parameters influence salmon production. Production would be increased by increasing stream survival or by decreasing the number of years spent in the stream (e.g. increasing MUG or decreasing MUL) (Fig. 8). If survival is high, production will be less sensitive to any other parameter (Fig. 9). (By survival, we refer here only to the SURV parameter in the model, which determines survival after the sea run of age 3 smolts. Smolt data do not reflect survival rates of young parr. If, as seems plausible, an increase in SURV were to be accompanied by an increase in the survival of young parr, the effect on yield would be even greater.) Again for the 1975 year-class we assessed how changes in each parameter influenced the number of smolt produced, from the starting 2000 parr. As each parameter was assessed, all other parameters were held constant at their "best" values. Neither of the variance parameters nor OFFSET appeared to alter productivity greatly over a wide range of parameter values (Fig. 8). Changes in growth rate or criterion length, on the other hand, both had large effects on smolt production. Effects of both parameters on productivity were nearly linear over the range of values observed in the various Western Arm Brook year-classes. Both high growth rates and low criterion lengths increase productivity. If true survival rates are higher than the "best" value of 0.40, productivity is higher and less sensitive to growth rate and smolt length whereas if true survival is lower, productivity is correspondingly lower and more sensitive to growth rate and smolt length (Fig. 9).

Discussion

To explain the observed pattern of smolt ages and lengths, it is necessary to modify Elson's (1957) idea of fixed length at smolting and assume that the criterion length for becoming a smolt decreases for older parr. This is a small step back towards the idea of fixed *age* at smolting. In addition, parr that smolt at older ages grow more slowly; thus, their potential for exceeding

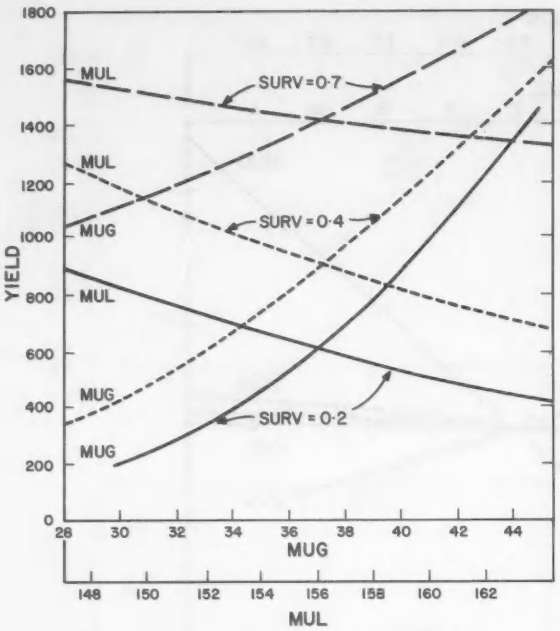

FIG. 9. Change in total yield (as in Fig. 7) and sensitivity of yield to MUG and MUL for SURV = 0.20, 0.40, and 0.70.

the criterion length is less. Nevertheless, the mean length of older smolts is greater. This is not a contradiction, but rather a consequence of the shapes of the distributions of growth rates of parr that will smolt at different ages. The growth rate distribution of age 3 smolts is concentrated near its lower limit and that of age 5 smolts near its upper limit (this is easiest to see by thinking of SIGL = 0, when the distributions are simply truncated portions of the underlying growth rate distribution (Fig. 1)).

Among the model parameters, SIGG, SIGL, and OFFSET are needed to let us fit the smolt data well, but their values do not have a great influence on smolt yield. It is conceivable that they may have an indirect effect by altering success at sea, but that is outside the scope of this work.

In trying to predict which parameters would be variable between year-classes, we expected that growth rate would probably be influenced by conditions within the stream, especially in the first year, but that criterion length had probalby evolved in response to expected conditions in the ocean and would be independent of stream conditions. In fact, we find roughly equal variability in 3·MUG and MUL (in the mean length and criterion length, respectively, of a 3+ parr) and in 4·MUG and MUL − OFFSET (the same for a 4+ parr). Therefore, we examined the possibility that both parameters reflected environmental variation. Temperature predicts the mean smolt age, or equivalently, the proportion of age 3 to age 4 smolts (Chadwick 1982). This proportion is set by both MUG and MUL, the two parameters that influence yield from the river, as well as by OFFSET. Temperature predicts none of the three parameters individually ($\tau_{TEMP \times MUG} = -0.286$; $\tau_{TEMP \times MUL} = 0.357$; $\tau_{TEMP \times OFFSET} = -0.071$; all $P > 0.10$). Similarly, we earlier showed that average size of age 4 smolts is significntly correlated with the growth rate parameter whereas average size of age 3 smolts is associated with the smolt length parameter. Sizes of these two age groups combine to produce the mean year-class length, which is uncorrelated with any model parameter. Hence, the greater inter-year-class variation in MUL and OFFSET than in MUG does not mean that factors related to criterion smolt length are necessarily more sensitive to environmental influences than are growth rates. Rather, the environment may influence the growth rate of a population of parr in its first year and apparently may also influence both the criterion lengths of 3- and 4-yr-old smolts, such that the final result (specific proportions of 3- and 4-yr-old smolts of certain sizes) reflects the environmental influences better than any single component of the system does. Because of these interactions among component processes (growth and smolting) only relatively subtle investigations are likely to be productive. In retrospect, it was suggestive that there was no correlation between back-calculated growth rate and our prime environmental indicator, temperature. The variability in MUL remains an intriguing finding.

We had not expected the model or the smolt data to provide much information about parr survival, yet for every year-class except 1969 the "best" set of parameters has SURV ≈ 0.40. The consistency is reassuring: we should have been hard pressed to think of a reason why survival rate should vary from year-class to year-class, depending on the year of birth but not the calendar year. It also supports the initial conjecture that density-dependent factors had little influence on Western Arm Brook parr. Year-class strength differed by more than a factor of two without affecting mortality rates. Because survival between ages within year-classes and among year-classes is constant, one can draw general conclusions about patterns of growth rate and smolt lengths among years and about the relationships of growth and smolting processes to salmon production.

The large size of acceptable parameter space for the 1975 year-class underscores two points. First, although our criterion for acceptance of the models was rigorous, when there is a good fit between model predictions and the data from Western Arm Brook salmon we cannot constrain tightly the likely growth rates, growth variances, or criterion lengths. Second, given the large size of acceptable parameter space for 1975 year-class with OFFSET intentionally kept at 0, the inability of the basic model to fit five year-classes at all, and to fit the other two year-classes only marginally, indicated a marked discrepancy between the model structure and the processes governing growth and smolting of parr. If a good fit is as liberal as we found for 1975 year-class, the discrepancy in model structure needed to prevent a fit must be large. In other words, the evidence is strong that the criterion smolt length decreases with age.

It is always easier to fit models to data if each parameter of the model has a different job to do. Much of our success in rejecting the original model came because one parameter, MUL, had to determine both the length of the shortest age 4 smolt and also the length of the longest age 4 smolt (any longer would have smolted at age 3). When we introduced OFFSET, it took over the first of these jobs. Although the revised model is elegant, and reveals a lot of originally unperceived regularity in the data, its success is perhaps not as surprising as it might seem.

Previous study led us to assume that parr in Western Arm Brook are not influenced by density-dependent factors (Chadwick 1982). Circumstantially, we infer that the model supports this assumption because good fits are consistently obtained with no density dependence present in the model. In this form, the model is useful for investigating environmental influences in

TABLE 8. Parameters providing acceptable fits for the 1976 and 1977 year-classes.

Year-class	MUG	SIGG	MUL	OFFSET	SIGL	SURV
1976	33.75	8.00	162	14	7.0	0.40
1977	33.0	9.00	155	11	7.0	0.40

salmon growth, smolting, and possibly survival. It may also act as a powerful tool for investigating density-dependent influences on these attributes of salmon by isolating specific failures of model predictions to fit data from other more heavily stocked systems.

Throughout this paper we have been treating Western Arm Brook like a black box, and looking only at its outputs. We justified this on the grounds that the outputs allowed much better sampling. However, it is clearly possible, and important, to study processes within the stream, i.e. study the parr themselves. Our work suggests that it is just as important to study influences on smolting as it is the more easily determined influences on growth rate. There are many ways to study salmon parr in Western Arm Brook. We have used very good data about a small piece of the story to obtain strong, general constraints on what the whole story can be like.

Coda

After the manuscript was otherwise completed, we analyzed the 1976 and 1977 year-classes in the same way. The model was able to fit both year-classes: the values of MUG were unusually low and those of SIGG were unusually high, but they were still reasonable (Table 8). We have not searched parameter space for the "best" values for these two year-classes, but all acceptable values of MUG will be low. Once again, fits with OFFSET = 0 were not possible. As we never examined these year-classes when we were formulating hypotheses, they serve as an independent check on the model.

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Appendix: Bounding the Space of Acceptable Parameter Sets

Pick a Year-Class and Set the Constraints

For a run of the model to be accepted as a representation of a year-class, it must satisfy the following three conditions (among others): (1) the proportion of age 4 smolts must be large enough, (2) the proportion of long age 3 smolts must be large enough, and (3) the proportion of long age 4 smolts must be small enough. We now translate these conditions into inequalities that the model parameters must satisfy.

1) Suppose that, in the chosen year-class, P age 3 smolts were observed, Q age 4, and R age 5 with $P + Q + R = X$ and that the model predicts p, q, and r, respectively ($p + q + r = X$). The G-statistic is

$$G = 2\left[P \ln \frac{P}{p} + Q \ln \frac{Q}{q} + R \ln \frac{R}{r}\right]$$

which must be less than 5.99 for acceptance at the 0.05 level. Let A be the shortfall in age 4 smolts: $A = Q - q$, the quantity that we do not want to become too large. Set $Y = p + r = P + R + A$. For fixed q, we minimize G by minimizing

$$F(p) = P \ln \frac{P}{p} + R \ln \frac{R}{Y - p}.$$

$$\frac{dF}{dp} = \frac{-P}{p} + \frac{R}{Y - p}$$

$$= 0 \text{ when } p = \frac{PY}{P + R},\ r = \frac{RY}{P + R}$$

i.e. when p and r are in the same proportion as P and R. We now have a formula for G as a function of A (and the observations):

$$G = 2\left[(P + R) \ln \frac{P + R}{P + R + A} + Q \ln \frac{Q}{Q - A}\right].$$

The solution of the equation $G = 5.99$, which can be obtained numerically, determines the largest tolerable value of A.

We can thus determine the flattest tolerable distribution of smolt ages consistent with what was observed, using flattest as a abbreviation for fewest age 4 smolts. It does not quite follow that we have determined the largest tolerable number of age 3 smolts, say; we could allow a few more age 3 smolts at the cost of many fewer age 5 smolts and some more age 4 smolts. In what follows, we shall nonetheless pretend that we have determined the largest tolerable number of age 3 and 5 smolts; this is almost true, and we prefer an uncluttered argument to strict theoremhood.

Dividing by the sampling intensities in different years, we get the flattest tolerable distribution of ages produced by the model. If we now assume a survival rate (there seems no way to infer one at this point), we can get the flattest tolerable potential smolt age distribution, i.e. the proportions of 3+ parr that will, but for mortality, smolt at ages 3, 4, and 5. This is the only place where an assumed survival rate enters the argument.

We end with two inequalities of the form

$$P(\text{smolt age} \leq 3) < \pi_3$$

$$P(\text{smolt age} \geq 5) < \pi_5$$

where the probabilities refer to the potential age distribution, relating length at age to criterion length, without taking survival into account (in effect, assuming SURV = 1.) We have assumed that all smolts age 3 or less are in fact age 3, and similarly, all smolts age 5 or greater are age 5. We shall carry this assumption through the argument; thus, in calculating the limits π_3 and π_5 we must take account of observed smolts of age 2 or 6.

2) The inequality concerning the length of age 3 smolts is much more direct. Given the number of age 3 smolts observed, we know the maximum deviation allowed before the K–S test will reject the model at the 0.05 level. Remember that we are treating the hypotheses as extrinsic. We must reject the model if the maximum deviation is exceeded at any length; to use our procedure, we choose the length that seems most likely to give trouble: typically a short length for the age 3 smolts and a long one for the age 4 smolts. Therefore, before we can accept the model, it must satisfy an inequality of the form (len (3): length at age 3; sa = smolt age)

$$P(\text{len}(3) > L_3 \mid \text{sa} \leqslant 3) > \lambda_3$$

where

$$\lambda_3 = 1 - (\text{observed } P + \text{maximum deviation}).$$

3) For age 4 smolts we work with the same sort of inequality, but on the other side:

$$P(\text{len}(4) > L_4 \mid \text{sa} = 4) < \lambda_4$$

where

$$\lambda_4 = 1 - (\text{observed } P - \text{maximum deviation}).$$

The model must satisfy many other inequalities as well; the interesting thing is that these four suffice to reject almost all the year-classes that cannot be fitted.

Infer Unconditional Probabilities from Conditional Ones

At this point we must pick probabilities

$$P_3 = P(\text{sa} \leqslant 3)$$

$$P_5 = P(\text{sa} \geqslant 5).$$

We generally choose them at their extreme values π_3 and π_5; exploring other possible values has not made fitting any easier. Define $P_4 = 1 - (P_3 + P_5) = P(\text{sa} = 4)$. By a standard result of probability theory,

$$P(\text{sa} \leqslant 3 \mid \text{len}(3) > L_3)\, P(\text{len}(3) > L_3) = P(\text{len}(3) > L_3 \mid \text{sa} \leqslant 3)\, P(\text{sa} \leqslant 3).$$

Also, $P(\text{sa} \leqslant 3/\text{len}(3) > L_3)$ is a probability, $\leqslant 1$. Therefore

$$P(\text{len}(3) > L_3) > \lambda_3 P_3.$$

Note that sa $\leqslant n$ means len (n) > criterion length (cl). By another standard result of probability theory:

$$\begin{aligned} P(\text{len}(4) > L_4) &= P(\text{len}(4) > L_4 \mid \text{sa} \leqslant 3)\, P(\text{sa} \leqslant 3) \\ &\quad + P(\text{len}(4) > L_4 \mid \text{sa} = 4)\, P(\text{sa} = 4) \\ &\quad + P(\text{len}(4) > L_4 \mid \text{sa} \geqslant 5)\, P(\text{sa} \geqslant 5) \\ &< 1 \cdot P_3 + \lambda_4 \cdot P_4 + P(\text{len}(4) > L_4 \mid \text{len}(4) < \text{cl}) \cdot P_5 \\ &< P_3 + \lambda_4 \cdot P_4 + \tfrac{1}{2} P_5, \text{ provided } L_4 > \text{MUL}. \end{aligned}$$

In general, L_4 will be significantly larger than MUL, so that the factor $\frac{1}{2}$ can be replaced by something much closer to 0.

To summarize, we have the following inequalities:

$$P(\text{len}(3) > \text{cl}) = P_3 < \pi_3$$

$$P(\text{len}(4) > \text{cl}) = 1 - P_5 > 1 - \pi_5$$

$$P(\text{len}(3) > L_3) > \lambda_3 \cdot P_3$$

$$P(\text{len}(4) > L_4) < P_3 + \lambda_4 \cdot P_4 + \tfrac{1}{2} P_5.$$

Interpret the Inequalities Inside the Probabilities

The growth rate of a parr is distributed as $N(\text{MUG}, \text{SIGG}^2)$. Therefore, the length of a 3+ parr, $\text{len}(3) \sim N(28 + 3 \cdot \text{MUG}, 9 \cdot \text{SIGG}^2)$, and

$$\text{len}(3) - L_3 \sim N(28 + 3 \cdot \text{MUG} - L_3, 9 \cdot \text{SIGG}^2).$$

Therefore

$$P(\text{len}(3) > L_3) = P\left[N(0, 1) > \frac{L_3 - 28}{3 \cdot \text{SIGG}} - 3 \cdot \text{MUG}\right].$$

So, $P(\text{len}(3) > L_3) > \lambda_3 \cdot P_3$ means

$$L_3 - 28 - 3 \cdot \text{MUG} < 3 \cdot \text{SIGG} \cdot \phi(\lambda_3 \cdot P_3)$$

where ϕ is the inverse Gaussian integral: $\phi(X)$ is that abscissa that has an area X of $N(0,1)$ to the right of it. Similarly,

$$P(\text{len}(4) > L_4) < P_3 + \lambda_4 P_4 + \tfrac{1}{2} P_5$$

means

$$L_4 - 28 - 4 \cdot \text{MUG} > 4 \cdot \text{SIGG} \cdot \phi(P_3 + \lambda_4 P_4 + \tfrac{1}{2} P_5).$$

Also, since the difference of two independent Gaussian variables is Gaussian (this explains why we chose a Gaussian rather than logistic model for length at smolting):

$$\text{len}(3) - \text{cl} \sim N(28 + 3 \cdot \text{MUG} - \text{MUL},\ 9 \cdot \text{SIGG}^2 + \text{SIGL}^2)$$

and $P(\text{sa} \leqslant 3) \leqslant P_3$ means

$$\text{MUL} - 28 - 3 \cdot \text{MUG} \geqslant (9 \cdot \text{SIGG}^2 + \text{SIGL}^2)^{1/2} \phi(P_3).$$

Assume $\phi(P_3) > 0$ (fewer than half the parr would smolt at age $\leqslant 3$; this is true for all year-classes for all plausible survival rates). Then

$$\text{MUL} - 28 - 3 \cdot \text{MUG} > 3 \cdot \text{SIGG} \cdot \phi(P_3).$$

Similarly, assuming $\phi(1 - P_5) < 0$,

$$\text{MUL} - 28 - 4 \cdot \text{MUG} < 4 \cdot \text{SIGG} \cdot \phi(1 - P_5).$$

To summarize, the model must produce the following:

few enough age 3 smolts:

(1) $\text{MUL} - 28 - 3 \cdot \text{MUG} > 3 \cdot \phi(P_3) \cdot \text{SIGG}$

few enough age 5 smolts:

(2) $\text{MUL} - 28 - 4 \cdot \text{MUG} < 4 \cdot \phi(1 - P_5) \cdot \text{SIGG}$

enough long age 3 smolts:

(3) $\dfrac{L_3 - 28}{3} - \text{MUG} < \phi(\lambda_3 \cdot P_3) \cdot \text{SIGG}$

enough short age 4 smolts:

(4) $\dfrac{L_4 - 28}{4} - \text{MUG} > \phi(P_3 + \lambda_4 P_4 + \tfrac{1}{2} P_5)\text{SIGG}.$

Rearrange the Inequalities

Equation 3 minus equation 4 gives

(5) $$\text{SIGG} > \frac{\dfrac{L_3 - 28}{3} - \dfrac{L_4 - 28}{4}}{\phi(\lambda_3 P_3) - \phi(P_3 + \lambda_4 P_4 + \tfrac{1}{2} P_5)}.$$

Equation 1 minus equation 2 gives

(6) $\text{MUG} > [3 \cdot \phi(P_3) - 4 \cdot \phi(1 - P_5)]\text{SIGG}.$

Equation 4 becomes

$$(7)\quad \mathrm{MUG} < \frac{L_4 - 28}{4} - \phi(P_3 + \lambda_4 P_4 + \tfrac{1}{2}P_5)\mathrm{SIGG}.$$

Equation 5 sets a lower bound on SIGG. Equation 6 sets a lower bound on MUG, which increases for larger SIGG. Equation 7 sets an upper bound on MUG, which either decreases for larger SIGG or, if it increases, does so more slowly than equation 6. If the bounds on MUG from equations 6 and 7, using the smallest value of SIGG consistent with equation 5, do not overlap, then we know that no set of parameters can produce a model that would be accepted as a representation of the given year-class.

If the bounds do overlap, redo the calculation replacing $P_3 + \lambda_4 P_4 + \frac{1}{2}P_5$ with $P_3 + \lambda_4 P_4$. If now the bounds do not overlap, then it may be possible to find an intermediate value to replace $\frac{1}{2}$, in a manner best described by example.

Example: the 1974 Year-Class

Observed numbers: 97 age 3, 89 age 4, 12 age 5, or $P = 97$, $Q = 89$, $R = 12$:

$$G = 2\,97 \ln\frac{97}{p} + 89 \ln\frac{89}{q} + 12 \ln\frac{12}{r}$$
$$= 1821.6 - [218 \ln A + 178 \ln (198 - A)].$$

If $A = 125.8$, then $G = 5.9$. So, the flattest acceptable distribution has $p = 112.0$, $q = 72.2$, $r = 13.8$. Correcting for sampling intensities, 1.78, 1.22, and 1.94%, respectively, the model should produce smolts in proportions no flatter than 62.9:59.2:7.1 or with SURV = 0.4, potential smolts in proportions no flatter than 62.9:148.0:45.1. So, $\pi_3 = 0.246$ and $\pi_5 = 0.176$. The maximum deviation from the age 3 length distribution allowed by the K–S test (for 97 observations) is 0.136 and for age 4 (89) is 0.142. We choose $L_3 = 169$ mm, proportion $\leq L_3 = 43/97$:

$$\lambda_3 = 1 - (43/97 + 0.136) = 0.421.$$

$L_4 = 180$ mm, proportion $\leq L_4 = 66/89$:

$$\lambda_4 = 1 - (66/89 - 0.142) = 0.400.$$

Choose

$$P_3 = \pi_3 = 0.246,\ \phi(P_3) = 0.687$$
$$P_5 = \pi_5 = 0.176,\ \phi(1 - P_5) = -0.931$$
$$\lambda_3 P_3 = 0.109,\ \phi(\lambda_3 P_3) = 1.232$$
$$P_3 + \lambda_4 P_4 + \tfrac{1}{2}P_5 = 0.565,\ \phi(P_3 + \lambda_4 P_4 + \tfrac{1}{2}P_5) = -0.164$$
$$P_3 + \lambda_4 P_4 = 0.477,\ \phi(P_3 + \lambda_4 P_4) = 0.057.$$

From equation 5:

$$(8)\quad \mathrm{SIGG} > \frac{47 - 38}{1.232 + 0.164} = 6.448$$

From equation 6:

$$(9)\quad \mathrm{MUG} > [3(0.687) + 4(0.931)]\cdot\mathrm{SIGG} > 37.34$$

From equation 7:

$$(10)\quad \mathrm{MUG} < 38 + 0.164\cdot\mathrm{SIGG} < 39.06$$

(taking the lowest value of SIGG. This is not a strict inequality, but it illustrates the existence of an overlap). Consider replacing $\frac{1}{2}P_5$ with something smaller: how much smaller would it have to be to eliminate the overlap (and thereby prove that the model cannot fit the 1974 year-class)? Reviewing equations 8–10, we want

$$5.792\cdot\mathrm{SIGG} > 38 - A\cdot\mathrm{SIGG}$$

where

$$\mathrm{SIGG} > 9/(1.232 - A).$$

Therefore

$$(5.792 + A)9 > 38(1.232 - A)$$
$$A > -0.113.$$

Therefore

$$P_3 + \lambda_4 P_4 + \alpha P_5 < \phi^{-1}(-0.113) = 0.545$$
$$0.176\cdot\alpha < 0.0677,\ \alpha < 0.385.$$

This means

$$(11)\quad P\,(\mathrm{len}\,(4) > L_4 \mid \mathrm{len}\,(4) < \mathrm{cl}) < 0.385.$$

If equation 11 is satisfied, then we can reject the model for the 1974 year-class. Now recall equation 2:

$$\mathrm{MUL} < 28 + 4\cdot\mathrm{MUG} + 4\cdot\phi(1 - P_5)\cdot\mathrm{SIGG}$$
$$< 28 + 4(39.056) - 4(0.931)(6.448) = 160.22.$$

Equation 11 is certainly satisfied if

$$P\,(\mathrm{len}\,(4) > L_4 \mid \mathrm{len}\,(4) = \mathrm{cl}) < 0.398$$

i.e. if

$$P\,(\mathrm{cl} > L_4) < 0.398.$$

Now,

$$\mathrm{cl} - L_4 \sim N(-19.78, \mathrm{SIGL}).$$

So, we want

$$P\left(N(0, 1) > \frac{19.78}{\mathrm{SIGL}}\right) < 0.385$$

$$\frac{19.78}{\mathrm{SIGL}} > 0.292,\ \mathrm{SIGL} < 67.74.$$

So, provided SIGL < 67.74, the model cannot fit the 1974 year-class. But if SIGL > 67.74, then equation 6 can be replaced with

$$\mathrm{MUG} > (9\cdot\mathrm{SIGG}^2 + \mathrm{SIGL}^2)^{1/2}\phi(P_3)$$
$$- (16\cdot\mathrm{SIGG}^2 + \mathrm{SIGL}^2)^{1/2}\phi(1 - P_5)$$
$$> 70.4(0.687) + 72.5(0.931) = 115.9$$

which is certainly incompatible with equation 7.

NOTES

Vertical Distribution of Atlantic Halibut (*Hippoglossus hippoglossus*) Eggs

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It is suggested that the vertical distribution of Atlantic halibut (*Hippoglossus hippoglossus*) eggs is determined by their specific density and that it is closely correlated to seawater salinity. In two deep North Norwegian fjords, only one halibut egg was found near the bottom (approximately 5200 m^3 seawater filtered), while 278 eggs were found floating pelagically in intermediate water layers (approximately 190 000 m^3 seawater filtered). Eggs were most abundant in water masses where temperature and salinity ranged between 4.5 and 7.0°C and 33.8 and 35.0‰. Neutral buoyancy salinity measurements of living eggs corresponded approximately with the observed capture salinities. Mean capture salinity was 34.2 ± 0.3‰ (Malangen) and 34.5 ± 0.4‰ (Sørøysund). Egg diameters ranged from 3.06 to 3.49 mm.

La distribution verticale des œufs de flétan atlantique (*Hippoglossus hippoglossus*) serait déterminée par leur densité spécifique et en étroite corrélation avec la salinité de l'eau de mer. Dans les fjords profonds du nord de la Norvège, on n'a trouvé qu'un seul œuf de flétan près du fond (approximativement 5200 m^3 d'eau de mer filtrée), tandis que 278 œufs flottant entre deux eaux ont été récupérés des couches intermédiaires (approximativement 190 000 m^3 d'eau de mer filtrée). Les œufs se trouvaient en plus grand nombre dans les masses d'eau de température de 4,5–7,0°C et de salinité de 33,8–35,0 ‰. Des mesures de salinité au point de flottabilité neutre d'œufs vivants correspondaient approximativement aux salinités observées aux endroits de capture. À ces derniers, la salinité était de 34,2 ± 0,3 ‰ (Malangen) et 34,5 ± 0,4 ‰ (Sørøysund). Le diamètre des œufs était compris entre 3,06 et 3,49 mm.

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The Atlantic halibut (*Hippoglossus hippoglossus*) is a subarctic flatfish, distributed on both sides of the North Atlantic. In the Northeast Atlantic it spawns in early winter in specific soft clay or mud bottom deepwater localities (300–700 m) in fjords and on the edge of the continental shelf along the Norwegian coast (Hjort 1905; Devold 1938).

Little research has been done in the field on the ecology of halibut eggs. Although artificially fertilized eggs with specific gravity lower than the seawater at the spawning sites have been recorded both by the present authors (unpubl. data) and by V. Øiestad (Inst. of Mar. Res., Directorate of Fisheries, Bergen, Norway, pers. comm.), artificially fertilized eggs are usually buoyant in salinities considerably above those recorded on the halibut spawning grounds (Lønning et al. 1982; Blaxter et al. 1983). Riis-Vestergaard (1982) found no osmotic or inorganic malfunction in the heavy type of halibut eggs; he found them to possess a low relative chorion weight, which is usually the main contributor to negative buoyancy, and concluded that a high content of organic matter in the rest of the egg was the cause of the negative buoyancy. One hypothesis could thus be that early halibut development takes place at or very near the seabed. The few existing records of halibut eggs in the sea (Fig. 1) suggest, however, that they are bathypelagic, probably floating at great depths (Rollefsen 1934; Vedel-Tåning 1936; Devold 1938, 1939, 1943; McIntyre 1958). The morphology and ultrastruc-

[1]Authorship equal.

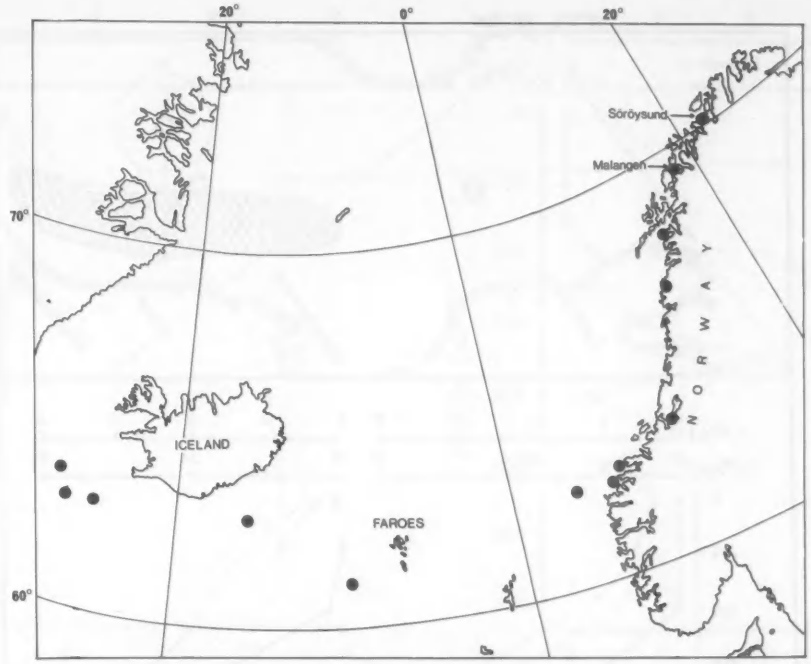

FIG. 1. Geographical position of the spawning grounds in Malangen and Sørøysund and the sites (●) where Atlantic halibut eggs have been recorded by previous investigators (see text for references).

ture of the eggs and the embryonic development also suggest that the early stages of the halibut are pelagic (Lønning et al. 1982).

To better understand the ecology of Atlantic halibut eggs and to clarify the contradictions between laboratory and field data, egg surveys were carried out on two halibut spawning grounds in North Norway during the spawning seasons of 1982 and 1983.

Material and Methods

The egg surveys were carried out in Malangen and Sørøysund, two well-known halibut spawning grounds in North Norway (Fig. 1). Halibut, both spent and with running milt and roe, caught at the same time as the egg surveys confirmed that spawning took place during the sampling periods.

In 1982, 39 horizontal hauls, each of 30 min, were made at different depths in the two localities using a Tucker trawl (mesh size 1.0 mm). The approximate volume filtered during each haul (1400 m^3) was calculated using the area of the trawl opening (1 m^2) and the cruising speed of the ship (1.5 knots). A Beyer's epibenthic closing net (Hesthagen 1970) was used to search for eggs on the seafloor (approximately 350 m depth) of the Malangen spawning ground. Ten hauls, each of 30 min and an approximate filtered volume of 225 m^3, were made using this equipment. Hydrographical data were recorded in the middle of the survey areas using a Neill Brown CTD-sonde connected to a Nord-10 computer. For a more detailed description of the CTD-sonde–computer system, the Tucker trawl, and the Simrad trawl eye used in these investigations, see Hopkins et al. (1982).

During the 1983 egg surveys in Malangen, a MOCNESS multiple opening–closing net and environmental sensing system (Wiebe et al. 1976) were used. Surveys were made at various depths by hauling the MOCNESS along a fixed line through the Malangen halibut spawning ground (Fig. 2). Each of the sampling nets was hauled for 30 or 60 min, and volumes filtered were calculated from flowmeter readings. Hydrographical data were recorded every 5 m using the CTD-sonde at two stations along the fixed hauling line (Fig. 2). After determining the horizontal and vertical distribution of the eggs along the fixed line, a closer examination of their vertical distribution was made in the area of highest egg concentrations. In this area, possible patchiness in egg distribution was examined by towing the MOCNESS at 150 m depth along the sides of a square, each side lasting 30 min, and two vertical profiles were determined by hauling the MOCNESS in approximately equal circles at various depths (Fig. 2). Hydrographical data were recorded every 5 m using the CTD-sonde in the middle part of the sampling circle between the sampling of the two vertical profiles.

The 1983 surveys in Sørøysund were carried out using the Tucker trawl approximately 2 wk after the Malangen surveys. Sixty-minute hauls were made at different depths from the surface to the bottom along a transect where the gill net fishery also took place. In the middle of the sampling period, which lasted approximately 24 h, hydrographical data were recorded every 5 m.

Halibut eggs were identified by their diameters (Russell 1976) immediately upon arrival on deck. In 1982, they were fixed in a solution of 2.5% glutaraldehyde and 2.5% formaldehyde in 0.05 mol/L cacodylate buffer (pH = 7.2, 350 mosmol/kg) (e.g. see Glauert 1974), as this fixative did not have any detectable effect on their diameter. After 12 h they were

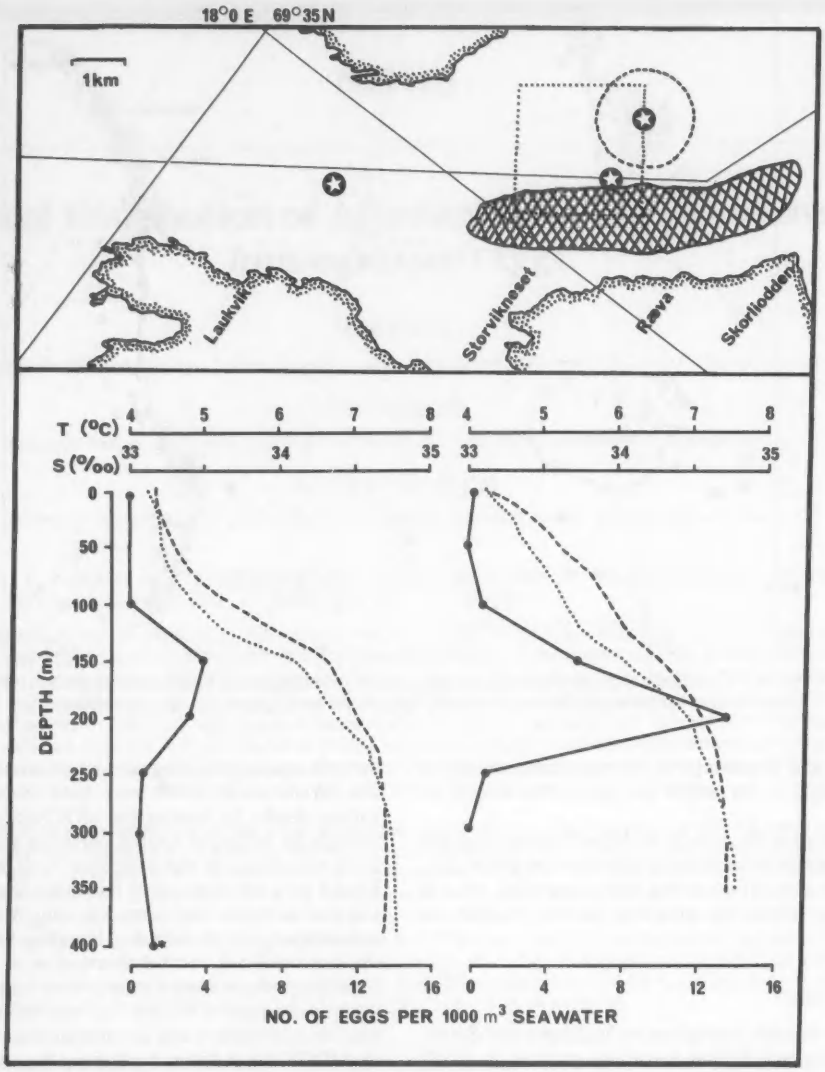

FIG. 2. Temperature (---), salinity (···), and calculated egg concentrations (—) at different depths between Laukvik and Storvikneset (left curves) and between Storvikneset and Skorliodden (right curves) on the Malangen spawning grounds in 1983. Bottom sample represented by black star. Positions of CTD stations (encircled stars) and localization of the "square" (···), and "circle" (---) hauls with the MOCNESS are also indicated on the map (see text for further explanation). The hatched area on the map shows where the gill net fishery took place.

transferred to 0.05 mol/L cacodylate buffer for storing and later determination of diameter and developmental stage. In 1983, many eggs were preserved as in 1982, but when possible, the developmental stage and diameter of live eggs were determined on board. These eggs were kept in jars containing fresh seawater and, as soon as possible, transferred to a density-gradient column (Coombs 1981; Pettersen and Køgeler 1983) where the neutral buoyancy salinity of each egg was determined. The egg stages were determined according to Rollefsen (1934) and Lønning et al. (1982). All plankton samples were preserved in 4% formalin in seawater and reinspected at the laboratory.

Results

Hydrography and egg distribution in Malangen — No halibut eggs were found in the 10 hauls (representing approximately 2250 m^3 of water) of the Beyer's epibenthic closing net. Mud, sand, and typical bottom-dwelling polychaetes and mussels in the samples confirmed that the net had sampled the bottom as intended. One MOCNESS haul was mistakenly carried out along the bottom at 400–420 m, and sand and mud in the nets left no doubt as to where sampling has taken place. During the 2 h of MOCNESS bottom sledging, 2951 m^3 of

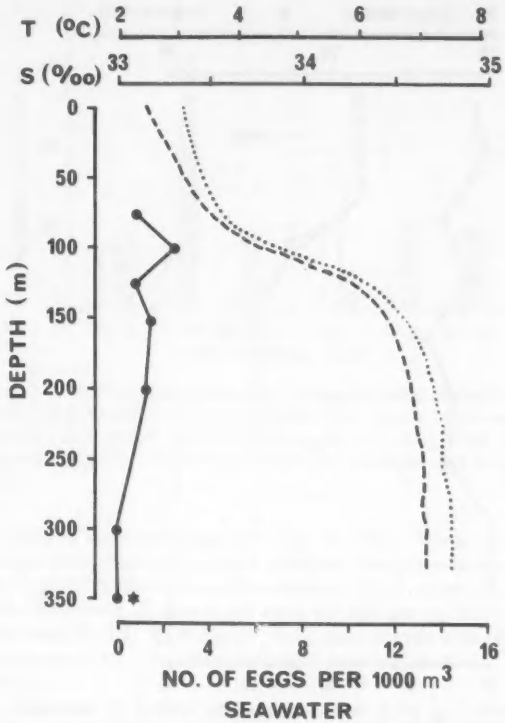

FIG. 3. Temperature (---), salinity (···), and calculated egg concentrations (—) at different depths in Malangen in 1982. Bottom sample represented by black star.

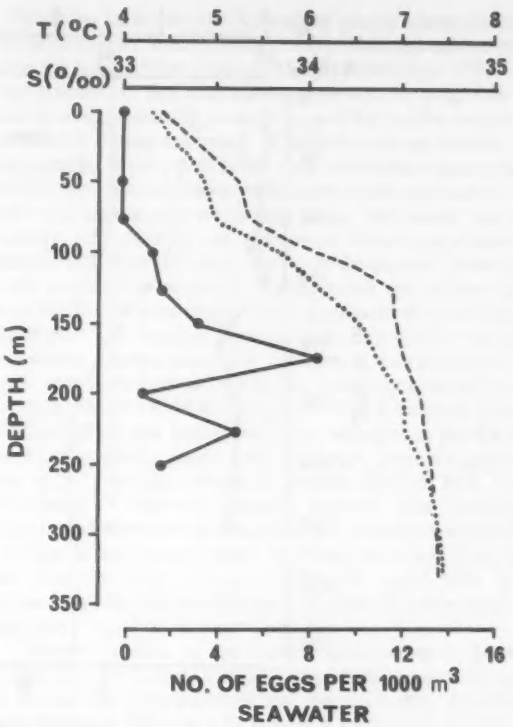

FIG. 4. Temperature (---), salinity (···), and calculated egg concentrations (—) at different depths in Malangen from the two 1983 MOCNESS circle samples (see text for further explanation).

near-bottom water was filtered, yielding only one dead halibut egg.

In 1982, 27 halibut eggs were caught in 16 Tucker trawl hauls. During the sampling, a layer of rapidly changing temperature and salinity (and thus also seawater density) was situated between depths of approximately 90 and 140 m (Fig. 3). Within this region, 10 and 3 eggs were found in three hauls each at 100 and 125 m depths, respectively. In the warmer and more saline lower layer, 6 and 5 eggs were caught in three hauls each at 150 and 200 m depths, respectively. Using the approximate Tucker trawl filtering volume of 1400 m^3/30 min, it appears that the largest egg concentration observed was 2.4 eggs/1000 m^3 seawater (100 m depth, salinity 33.85‰).

Hydrographical data recorded during the 1983 sampling period suggested both day-to-day changes and local differences in the hydrographical conditions due to water movements. The CTD recordings given in Fig. 2 and 3 reveal a less defined region of temperature and salinity changes with respect to depth than in 1982. The 1983 salinity and temperature increased most rapidly at depths less than 200–250 m. Below 250 m, the salinity increased very little, as did the temperature below 200 m.

One hundred and ninety eggs were caught during the 1983 MOCNESS surveys in Malangen. The amount of seawater filtered differed at the various depths, as some depths were surveyed more intensively in areas of expected high egg concentrations. During the hauls along the fixed line shown in Fig. 2, few eggs were caught except in hauls at 150 and 200 m. The largest concentration of eggs was found in the areas outside Ræva between Skorliodden and Storvikneset at 200 m (13.92 eggs/1000 m^3 seawater). Hauls in the vicinity of the areas shown in Fig. 3 yielded very few eggs except at 150 m depth where egg concentrations of 6.25 and 3.24 eggs/1000 m^3 seawater were found northwest of Laukvik and southeast of Skorliodden, respectively. Since the egg concentration at 150 m depth between Skorliodden and Storvikneset was nearly the same (5.81 eggs/1000 m^3 seawater) as that northwest of Laukvik, the former area was regarded as most suitable for the subsequent vertical profile determinations. Furthermore, the square hauls at 150 m in various parts of this area (Fig. 2) revealed higher concentrations (6.70 and 3.17 eggs/1000 m^3 seawater) in the two easternmost hauls, while the two hauls to the west yielded only 0 and 1.63 eggs/1000 m^3 seawater. Repeated examination of the vertical distribution of eggs in the area of highest egg concentration further confirmed the findings that egg concentrations were highest at depths between 150 and 225 m, most eggs being found at 175 m with 8.39 eggs/1000 m^3 seawater (Fig. 4).

Hydrography and egg distribution in Sørøysund — In 1982, a layer of rapidly changing water salinity and temperature was located between 120 and 260 m depths during the sampling period (Fig. 5). Of 26 halibut eggs collected in 23 hauls, very few were found in the upper cold and low-salinity layer. Hauls within the region of rapidly changing water density yielded most eggs, as three hauls at each of depths 200 and 250 m resulted in 10 and 6 eggs, respectively.

In 1983, the water temperature was considerably higher than

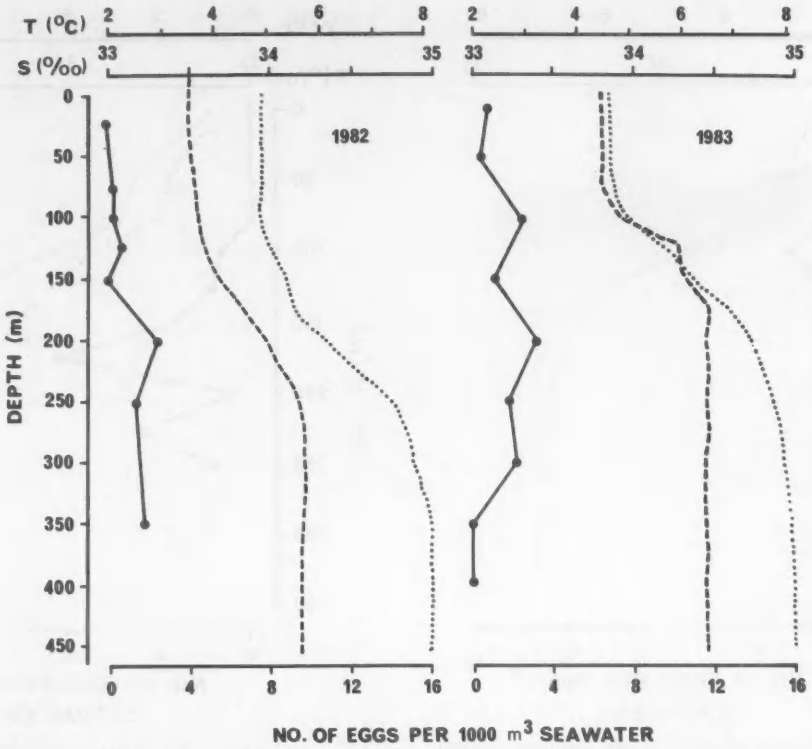

FIG. 5. Temperature (---), salinity (· · ·), and calculated egg concentrations (—) at various depths on the Sørøysund spawning ground in 1982 and 1983.

in 1982 (Fig. 5). There was almost no change in salinity between the surface and 100 m. A region of more rapidly changing salinity with depth (although not as rapid as in 1982) was located between approximately 100 and 250 m depths, whereas there was only a minor increase in salinity below 250 m depth. Thirty-five eggs were caught in nine Tucker trawl hauls (60 min each) (Fig. 5). Most eggs were present at depths between 100 and 300 m, the highest number (9) being found at 200 m depth. Bearing in mind the uncertainty of the filtering volume determinations, a rough calculation of egg concentration revealed maxima of 2.4 and 3.2 eggs/1000 m^3 seawater at 200 m depth in 1982 and 1983, respectively. Salinities at 200 m were 34.4‰ (1982) and 34.7‰ (1983).

Egg densities, developmental stages, and diameters — There was a general increase in neutral buoyancy salinity of eggs with increasing capture salinity (Fig. 6). Both in Malangen and Sørøysund, the least dense eggs were collected at 0–100 m (capture salinities 33.2–33.9‰), while eggs with neutral buoyancy salinities ranging between 34.0 and 35.0‰ were found at greater depths. The range in neutral buoyancy salinities of eggs captured from equal salinities was considerable, particularly for eggs collected in deeper water, and neutral buoyancy salinities determined experimentally were generally slightly lower than the capture salinities (Fig. 6).

Calculated mean capture salinity for eggs from Malangen was 34.2 ± 0.3‰ and for eggs from Sørøysund was 34.5 ± 0.4‰. There was no significant difference (t-test, $P > 0.05$) in mean capture salinity between sampling years (1982 and 1983) within

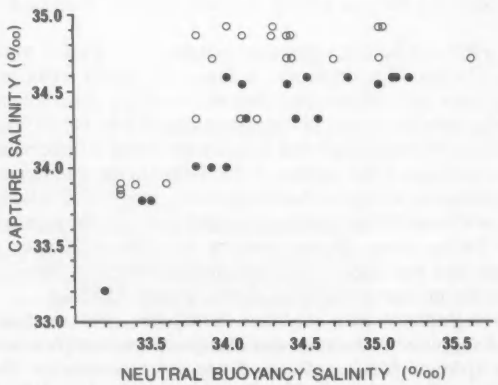

FIG. 6. Relation between measured neutral buoyancy salinity and capture salinity (determined from CTD data) for halibut eggs caught in Malangen (●) and Sørøysund (○).

any of the areas. On the other hand, the difference in capture salinity (1982 and 1983 materials pooled) between Malangen and Sørøysund was highly significant (t-test, $P < 0.01$).

Unfortunately, many eggs were cytolyzed after capture, such that their developmental stages could not be determined. In 1982, most of the eggs whose developmental stage could be determined were either blastulae or early gastrulae, i.e. stages younger than 6 d. In 1983, the majority of eggs had visible embryos and were thus older than 6 d. This difference was

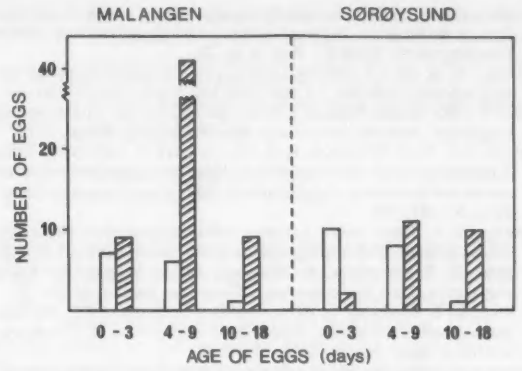

FIG. 7. Developmental stages of halibut eggs found in Malangen and Sørøysund: blastulae (0–3 d), gastrulae until closure of blastopore (4–9 d), and embryo with closed blastopore (10–18 d). White areas represent eggs found in 1982; hatched areas represent eggs found in 1983.

evident at both sampling sites (Fig. 7). Most developmental stages were found in all capture salinities. This was also true for the eggs in the density-gradient column. There was no significant difference in density between the age groups that were blastulae (0–3 d), gastrulae (4–9 d), and embryos with closed blastopores (10–18 d) from Malangen (one-way ANOVA, $P > 0.05$).

Diameters of halibut eggs ranged from 3.06 to 3.49 mm. Mean egg diameters (1982 and 1983 data pooled) in Malangen and Sørøysund were 3.23 ± 0.08 and 3.18 ± 0.07 mm, respectively. The difference between the two areas was statistically significant (t-test, $P < 0.01$). However, there were also significant differences (t-tests, $P < 0.05$) observed within each locality between sampling years.

Discussion

Contrary to the view of Vedel-Tåning (1936), who considered March–May to be the main spawning period for Northeast Atlantic halibut, all reports from the Norwegian coast suggest that halibut spawn in December–February (Hjort 1905; Devold 1938). The two areas investigated here are well-known spawning areas where halibut concentrate every autumn. Spawning in Malangen usually starts around Christmas (Devold 1938), while Tjemsland (1960) reported a peak spawning period in Sørøysund from approximately January 20 to February 15. The present study confirms that spawning occurs in January and February in both the investigated areas. A larger proportion of late developmental stages were found in 1983 than in the same period of 1982. However, the water temperatures were higher in 1983 than in 1982 in both areas, thus making it difficult to determine whether the more advanced developmental stages in 1983 are the result of earlier spawning or a faster egg development.

In both years the surveys in Sørøysund were carried out later than in Malangen, especially in 1983. Assuming a similar spawning curve for both areas, the eggs collected in Sørøysund were spawned later than those taken in Malangen. Fish species with long spawning periods are known to produce smaller eggs at the end of the spawning period (Hiemstra 1962). The difference in egg size between the two localities may therefore be due to samples being taken at different stages of the spawning period.

Spawning takes place in Norwegian coastal waters down to depths of at least 700 m (Devold 1938). Although halibut often leave the bottom when chasing their prey (McIntyre 1953), the large numbers of fish with running milt and roe caught on the bottom on the spawning grounds suggest that halibut remain on the bottom during spawning. If halibut eggs are benthic, as suggested by Blaxter et al. (1983), one would then expect to find them in large concentrations on the seabed in the same areas. No such concentrations were found during this study. On the contrary, the majority of eggs were found suspended in intermediate layers of deep water. In Sørøysund, where the water column was relatively homogeneous and deeper, eggs were distributed more evenly over a greater depth range than in Malangen. This suggests that not only egg density but also convective currents determine the vertical distribution of the eggs. This view is supported by the density determinations of the eggs, which revealed a lower correlation between capture depth salinities and neutral buoyancy salinities in the deeper, more homogeneous layers where mixing is more conspicuous than in the pycnocline above (S. Sundby, Inst. of Mar. Res., Directorate of Fisheries, Bergen, Norway, pers. commn.). Since no increase in the abundance of the youngest developmental stages with increasing depth was observed, it is unlikely that the observed range of egg distribution could have been erroneously extended towards high salinities by newly spawned eggs ascending to their neutral salinity.

Comparative data on the vertical distribution of Atlantic halibut eggs are scarce. Vedel-Tåning (1936) reported 10 eggs of this species from Icelandic and Faroese waters distributed over depths of 700 m at a locality of a total depth of 1975 m, while McIntyre (1958) found 34 eggs floating mesopelagically at depths between 400 and 600 m in the Denmark Strait. A comparison between the Atlantic and the closely related Pacific halibut (*Hippoglossus stenolepis*) reveals close correspondence with respect to egg density, vertical distribution, and concentration (Thompson and van Cleve 1936; van Cleve and Seymour 1953). Data given in Thompson and van Cleve (1936, fig. 55), and converted to numbers of eggs/1000 m^3, indicate that highest egg concentrations were found in depths between 100 and 200 m in seawater densities of 1.0254–1.0266 g/cm^3. This is very similar to the present data from Malangen and Sørøysund. In the Pacific, a tendency to an increased egg concentration in the deepest layers was found. This may be a result of newly spawned eggs ascending to the upper layers.

All eggs collected during these investigations were found floating in salinities below 35.0‰. These salinities are considerably lower than the neutral buoyancy salinities of 35.5–37.0‰ found in stripped, artificially fertilized, and laboratory-reared halibut eggs (Lønning et al. 1982; Blaxter et al. 1983). In the Pacific halibut, egg density determinations from stripped fish indicated neutral buoyancy at a salinity of 31.5‰, which corresponded well with their mesopelagic distribution in nature (Forrester and Alderdice 1973). According to our own unpublished data and V. Øiestad (Inst. of Mar. Res., Directorate of Fisheries, Bergen, Norway, pers. comm.), eggs stripped from Atlantic halibut females have only occasionally been buoyant in salinities similar to those recorded at the spawning places. As shown by Rosenthal and Alderdice (1976), gonadal tissue and early embryos are especially susceptible to environmental stress, which may result in embryonic malfunctions and malformations. All rearing experiments with the Atlantic halibut have so far been carried out by stripping fish newly caught by gill net with running milt and roe, while in the Pacific

halibut experiments, fish that have been kept in captivity for 21 mo and were anaesthetized before stripping were used (Tomlinson and Baker 1973). The artificially fertilized eggs from the Atlantic halibut may therefore have been of varying quality. Cod (*Gadus morhua*) eggs of poor quality have higher densities than good-quality eggs, even though they may seem to develop and osmoregulate normally (Kjørsvik et al. 1984). The observed density differences between natural and artificially spawned eggs may therefore be due to factors such as the rough treatment given to mature fish caught in gill nets, the rapid ascent from depths of 350 m or more to the surface, and the mechanical effects of stripping on newly caught fish.

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A Medium for the Isolation and Differentiation of *Yersinia ruckeri*[1]

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Waltman, W. D., and E. B. Shotts. 1984. A medium for the isolation and differentiation of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. 41: 804–806.

A new medium, Shotts–Waltman (SW), was developed for the isolation and differentiation of *Yersinia ruckeri*; *Y. ruckeri* was distinguished by its ability to hydrolyze Tween 80 and inability to ferment sucrose. SW was shown to be as sensitive as MacConkey and trypticase soy agars for the growth of *Y. ruckeri*. SW is inexpensive, prepared easily, and should greatly increase the recovery of *Y. ruckeri* from clinical material and infected fish.

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Nous avons mis au point un nouveau milieu de culture, Shotts–Waltman (SW), permettant d'isoler et de différencier *Yersinia ruckeri*; ce dernier se distingue par son aptitude à hydrolyser le Tween 80 et son incapacité à fermenter le sucrose. Nous démontrons que SW est aussi sensible que les agars de MacConkey et soya trypticase pour la croissance de *Y. ruckeri*. Le milieu SW est peu dispendieux, facile à préparer et devrait permettre d'accroître considérablement la récupération de *Y. ruckeri* de matériel clinique et de poissons infectés.

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Yersinia ruckeri was first isolated in the early 1950s in the Hagerman Valley of Idaho as a result of mortality in hatchery-reared rainbow trout (*Salmo gairdneri*). The bacterium was first described by Ross et al. (1966) and Rucker (1966) as the causative agent of an enteric redmouth disease of salmonids. Ewing et al. (1978) classified this organism as a member of the Enterobacteriaceae and named it *Yersinia ruckeri*; formerly it had been referred to as either the Hagerman redmouth (HRM) bacterium or the enteric redmouth (ERM) bacterium.

Enteric redmouth disease is an acute to chronic bacteremic infection of salmonids that can result in 30–70% mortality. Transmission is horizontally facilitated by contact with asymptomatic carriers. Busch and Lingg (1975) reported that as many as 25% of rainbow trout could carry *Y. ruckeri* in their lower intestine with periodic shedding. Obviously, then, the disease may be spread by the movement of carrier fish from one locale to another. This has prompted a certification program for fish prior to transfer.

Diagnosis of enteric redmouth disease may include isolating *Y. ruckeri* either from clinically diseased fish or from asymptomatic carriers. Currently, diagnosis is accomplished by serological or cultural methods. Serologic detection is somewhat limited by the presence of cross reactivity (Bullock et al. 1978; Busch 1982). Culture techniques use primarily non-selective, non-differentiating agar media leading to difficulty in the isolation and identification of *Y. ruckeri*, especially from mixed populations.

Our objective was the development of a differential medium to isolate *Y. ruckeri*. Such a medium would greatly enhance diagnostic credibility and the ease with which *Y. ruckeri* can be recovered from mixed populations.

Materials and Methods

Sixty bacterial isolates culturally and serologically identified as *Y. ruckeri* were obtained from the National Fish Disease Research Laboratories, U.S. Fish and Wildlife Service at Fort Morgan, CO, and Kearneysville, WV. These isolates were examined for their ability to utilize a number of biochemical substrates including the hydrolysis of Tween 80 and the fermentation of sucrose (W. D. Waltman and E. B. Shotts. 1983. Eighth Annual Eastern Fish Health Workshop, Leetown, WV, June 21–23, 1983 (Abstr.)).

A wide variety of other microorganisms representing those genera frequently associated with aquatic environments were used to evaluate this medium and included selected species from the following genera: *Aeromonas*, *Serratia*, *Proteus*, *Yersinia*, *Hafnia*, *Enterobacter*, *Klebsiella*, *Escherichia*, *Edwardsiella*, *Plesiomonas*, *Citrobacter*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Erysipelothrix*, and *Corynebacterium*. The identity of all microorganisms used was verified by biochemical characterization (Breed et al. 1957).

A medium referred to as Shotts–Waltman (SW) was formulated. It was composed of Bacto-tryptone, 2 g; yeast extract, 2 g; Tween 80, 10 mL; sodium chloride, 5 g; calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.1 g; brom thymol blue, 0.003 g; agar, 15 g; and distilled water, 980 mL. Components were dissolved and the pH adjusted to 7.4. After autoclaving, the medium was cooled to 50°C and 10 mL of 0.5 g/mL filter-sterilized (0.45 μm) sucrose added. The plates were poured and refrigerated until use.

Other media used included MacConkey (MAC) and trypticase soy (TSA) agars and brain heart infusion (BHI) broth. These media were obtained commercially and were prepared as recommended by the manufacturer.

The SW medium was evaluated for efficacy by three methods. First, pure cultures of various bacteria were grown in BHI broth and plated onto SW to determine colonial characteristics. Second, mixed cultures of *Y. ruckeri* and other enteric and aquatic bacteria were prepared by combining equal parts of the respective broth cultures and plating this suspension onto SW to determine differentiation capabilities. Third, the sensitivity of SW was determined by suspending logarithmic dilutions of *Y. ruckeri* in physiological saline with subsequent streaking onto triplicate plates of SW, TSA, and MAC for quantitation. Counts obtained were averaged and means of five replicate studies were calculated. Differences in the means obtained were evaluated by Duncan's multiple range test.

Results

Initial studies showed that 53 of the 60 *Y. ruckeri* isolates (88.3%) were capable of hydrolyzing Tween 80, whereas none fermented sucrose. Additional investigation indicated that the seven Tween 80 nonhydrolyzing isolates gave other "atypical" biochemical reactions (W. D. Waltman and E. B. Shotts. 1983. Eighth Annual Eastern Fish Health Workshop, Leetown, WV, June 21–23, 1983 (Abstr.)).

Four possible reactions occurred in response to the combination of Tween 80 and sucrose in the SW plate (Table 1). An organism such as *Y. ruckeri* hydrolyzed Tween 80 but did not ferment sucrose, resulting in a green colony with a zone of hydrolysis. An organism such as *A. hydrophila* hydrolyzed Tween 80 and fermented sucrose, producing a yellow colony with a zone of hydrolysis. *Enterobacter* did not hydrolyze Tween 80 but fermented sucrose, as demonstrated by a yellow colony without a zone of hydrolysis. Finally, an organism such as *E. tarda* did not hydrolyze Tween 80 or ferment sucrose, giving a green colony without a hydrolytic zone. Reversion (alkalinization of the medium) occurred with some bacteria as a result of prolonged incubation. The ideal incubation conditions were found to be 30°C for 24–36 h.

Plating of mixed cultures showed that *Y. ruckeri* could be easily differentiated from other bacteria on the basis of colonial morphology and substrate utilization of SW (Table 1).

The sensitivity of SW as compared with MAC and TSA, the media that are currently available for isolation, is shown in

TABLE 1. Categorization of the reactivity of commonly encountered aquatic bacteria to Tween 80 and sucrose.

Sucrose fermentation	Tween 80 hydrolysis	
	Positive	Negative
Positive	*Aeromonas hydrophila* *Serratia* spp. *Proteus vulgaris* *Yersinia enterocolitica*	*Aeromonas hydrophila* *Hafnia alvei* *Enterobacter* spp. *Klebsiella* spp. Gram positive bacteria
Negative	*Yersinia ruckeri*	*Hafnia alvei* *Edwardsiella tarda* *Edwardsiella ictaluri* *Plesiomonas shigelloides* *Proteus* spp. *Citrobacter freundii* *Salmonella* spp. *Escherichia coli*

TABLE 2. Comparative sensitivity of *Yersinia ruckeri* growth on Shotts-Waltman (SW), MacConkey (MAC), and trypticase soy (TSA) agars.

Test	SW	MAC	TSA
1	154.7[a]	92.3	157.7
2	87.0	76.3	92.7
3	43.2	40.8	42.0
4	101.0	96.0	121.0
5	38.0	38.0	45.0
Mean	84.8	68.7	91.7

[a]Mean of triplicate plate counts.

Table 2. No significant differences between these three media were observed at a 95% confidence level.

Discussion

Because *Y. ruckeri* can hydrolyze Tween 80 but cannot ferment sucrose this provides a basis of differentiation from other commonly associated bacteria. The incorporation of Tween 80 into SW is facilitated by its solubility and heat stability. Hydrolysis of Tween 80 is visualized by the formation of a zone consisting of calcium–oleate complexes around the colony (Stanier et al. 1966), hence the necessity of having $CaCl_2$ in the media. Filter sterilization is recommended because of potential breakdown of the disaccharide sucrose when autoclaved. The pH of SW was evaluated from pH 7.0 to 8.0 and found to be optimal at pH 7.4 (±0.1), which results in a green-colored agar. The fermentation of sucrose is evident by the formation of yellow colonies due to acid production and the concurrent change of color in the pH indicator. Failure to ferment this sugar results in clear to green colonies. *Yersinia ruckeri* appears as pinpoint colonies that may reach 1–2 mm after prolonged incubation. These colonies are haloed by 3- to 4-mm zone of hydrolysis. *Yersinia ruckeri* is easily differentiated from *A. hydrophila* because the latter produces yellow 2- to 4-mm colonies with or without zones of hydrolysis and from *H. alvei* because the latter produces clear-green 1- to 3-mm colonies without zones of hydrolysis.

The sensitivity of SW was not found to differ significantly from that of either MAC or TSA.

In conclusion, SW is an inexpensive and easily prepared medium capable of differentiating *Y. ruckeri* on initial plating of clinical material. The use of SW should greatly increase the recovery of *Y. ruckeri* from clinical material and carrier fish and reduce the time and effort involved. SW should facilitate a better understanding of the epidemiology of transmission and of the carrier state in fish infected with *Y. ruckeri*.

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Characteristics of a Previously Unidentified Virus from Channel Catfish (*Ictalurus punctatus*)

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A virus (strain F82-34) was isolated from channel catfish (*Ictalurus punctatus*) that was clearly distinct from channel catfish virus (CCV). The virus induced extensive syncytia in channel catfish ovary (CCO) cells incubated at 26°C. The BB and CHSE-214 cell lines supported virus replication but the FHM line was refractory. Virus replication was optimal at 25°C where, after 96 h, approximately 50% of the virus was found to be released from CCO cells. Virus infectivity was stable for 1 mo at 4, 16, and 23°C and for 5 mo at −20 and −80°C but was lost after 7 d at 45°C. Partially purified virions are double-shelled hexagonal capsids characteristic of reoviruses and have an average diameter of 75 nm. F82-34 was related to but distinct from two other reoviruses from fish in neutralization studies; these three viruses, and possibly a fourth from American oysters (*Crassostrea virginica*), appear to represent a new taxonomic group within the family Reoviridae. We therefore propose that this new virus be tentatively designated catfish reovirus (CRV).

Un virus (lignée F82-34) a été isolé de la barbue de rivière (*Ictalurus punctatus*), nettement distinct de celui que l'on connaissait sous le nom de virus de la barbue de rivière (CCV). Le premier provoque des syncities considérables des cellules ovariennes de cette espèce (CCO) incubée à 26°C. Les lignées cellulaires BB et CHSE-214 supportent la réplication du virus tandis que la lignée FHM est réfractaire. Cette réplication est optimale à 25°C tandis qu'après 96 h, on a constaté qu'environ 50 % du virus avaient été libérés des cellules de CCO. Le pouvoir infectieux du virus est stable pendant 1 mo à 4, 16 et 23°C, pendant 5 mo à −20 et −80°C, mais à 45°C on l'a perdu après 7 jours. Les virions partiellement purifiés sont des capsides hexagonaux à double enveloppe caractéristiques des réovirus et ont un diamètre moyen de 75 nm. F82-34 est apparenté à deux autres réovirus isolés de poissons au cours d'études de neutralisation, mais il en est distinct ; ces trois virus, et possiblement un quatrième provenant de l'huître américaine (*Crassostrea virginica*), semblent représenter un nouveau groupe taxonomique au sein de la famille des Reoviridae. Pour cette raison, nous proposons pour ce nouveau virus le nom provisoire de réovirus de la barbue de rivière (CRV).

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Channel catfish virus (CCV) is the only known viral pathogen of channel catfish (*Ictalurus punctatus*), and severe mortality is associated with certain outbreaks (Plumb 1978). CCV has been isolated from channel catfish at three farms in Southern California. Following stock destruction and sanitation procedures, a monitoring program was established to examine mortalities at these and several other farms to determine if CCV had been eradicated. During the examination of fish undergoing a chronic mortality (<5%) at a farm where CCV had not been previously found, a new virus was detected. The biological and physical characteristics of this virus were clearly different from CCV. In this paper we describe some of the properties of this previously unidentified virus (reference strain F82-34) that infects channel catfish.

Materials and Methods

Virus isolation — The kidney, spleen, and liver of juvenile channel catfish (5 g) were pooled and processed as described by McDaniel (1979). Microbial contamination was elimated by treatment with antibiotics (Amend and Pietsch 1972) prior to inoculation of selected cell cultures.

Cell lines — The following cell lines were used in this study: channel catfish ovary, CCO (Bowser and Plumb 1980), brown bullhead (*Ictalurus nebulosus*), BB (Wolf and Quimby 1969), chinook salmon (*Oncorhynchus tshawytscha*), CHSE-214 (Fryer et al. 1965), and the fathead minnow (*Pimephales promelas*), FHM (Gravell and Malsberger 1965). All four cell lines were maintained in minimal essential medium (MEM, Flow Laboratories Inc.) with Earle's salts supplemented with 5 or 10% fetal calf serum (Sterile Systems Inc.), 100 IU/mL penicillin – 100 μg/mL streptomycin (Gibco Inc.), and 0.29 mg/mL glutamine (Flow Laboratories Inc.). The FHM, CCO, and BB cells were grown at 26°C. The CHSE-214 line was routinely propagated at 20°C except during virus titrations when the temperature was increased to 26°C.

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Virus titrations — The concentrations of virus in suspensions prepared from fish tissues or cell culture fluids were titrated following serial 10-fold dilutions in 96 well dishes (Falcon). The $\log_{10}$ 50% tissue culture endpoints ($TCID_{50}$) were then calculated by the method of Reed and Muench (1938) following 7 d of incubation at 26°C in CCO, BB, FHM, or CHSE-214 cells.

Serological comparisons — The serological relationship of F82-34 to CCV and two reoviruses from chum salmon (*Oncorhynchus keta*), CSV (Winton et al. 1981), and golden shiners (*Notemigonus crysoleucas*), GSV (Plumb et al. 1979) was examined. Sera was prepared in rabbits by inoculation of partially purified F82-34 virus. New Zealand white rabbits were injected subcutaneously in the back with an emulsion of virus and Freunds' complete adjuvant. After 4 wk the rabbits were again injected with 0.5 mL of virus in the marginal ear vein. Serum was collected from blood taken 7 d after the final injections and stored at −80°C until used. The serological relationships of F82-34, GSV, and CSV were compared following incubation of each virus with a 1:100 dilution of anti-F82-34 rabbit serum in MEM-5. The amount of virus infectivity remaining after 1 h of incubation in normal and anti-F82-34 rabbit sera was determined by $TCID_{50}$ analysis.

The indirect fluorescent antibody (IFA) test using fluorescein isothiocyanate conjugated goat anti-rabbit serum (Tago, Inc.) was also used to determine any antigenic cross reactivity between CCV- and F82-34-infected cell monolayers.

Temperature studies — The effect of temperature on the replication and stability of F82-34 was examined. Monolayers of CCO cells in 35-mm dishes were infected with F82-34 at a multiplicity of 0.01 $TCID_{50}$/cell. After a 1-h adsorption period the inoculum was removed and the cells rinsed three times with MEM without serum (MEM-0). Two millilitres of MEM with 5% serum (MEM-5) and buffered with 15 mmol/L *N*-2-hydroxyethyl-piperazine-*N'*-2-ethansulfonic acid (HEPES) was then added to each dish. Replicate dishes were placed at 15, 20, 25, 30, 35, and 40°C for further incubation. Virus released into the culture medium (cell-free virus, CFV) was collected over a period of 96 h and stored at −70°C. The concentrations of cell-associated virus (CAV) at 25°*C* were also determined by rinsing the cell sheets three times with MEM-0 and then scraping them into suspension with a rubber policeman and 1.0 mL of fresh MEM-5. Virus was released from the cells by three rapid freeze–thaw cycles. The concentrations of CAV and CFV were obtained by $TCID_{50}$ analysis on CCO cells.

The stability of F82-34 at 4, 16, 23, 35, and 45°C was determined by incubating replicate 0.1-mL aliquots of virus in MEM-5 and assaying for infectivity.

Results

Cytopathology — The cytopathic effect (CPE) of F82-34 in CCO cells was characterized by focal areas of syncytia (Fig. 1). Initially, these involved two to three nuclei (12 h postinoculation, PI) but eventually syncytia containing up to 20 or more nuclei were observed. After 36 h, cytoplasmic bridges began to form between syncytia and by 48 h PI at 26°C, the cells began to roll up and detach from the substrate.

Cell line sensitivity — The CCO, BB, FHM, and CHSE-214 cell lines grown in 25-cm^2 flasks were infected with F82-34 at a multiplicity of infection (MOI) of 0.1 $TCID_{50}$/cell. Characteristic CPE was observed after 48 h in both the CCO and BB lines at 26°C. Focal areas of CPE were observed in CHSE-214 cells after several days of incubation at 26°C but did not progress to involve the entire cell sheet. The FHM line did not demonstrate CPE up to 2 wk following infection at 26°C.

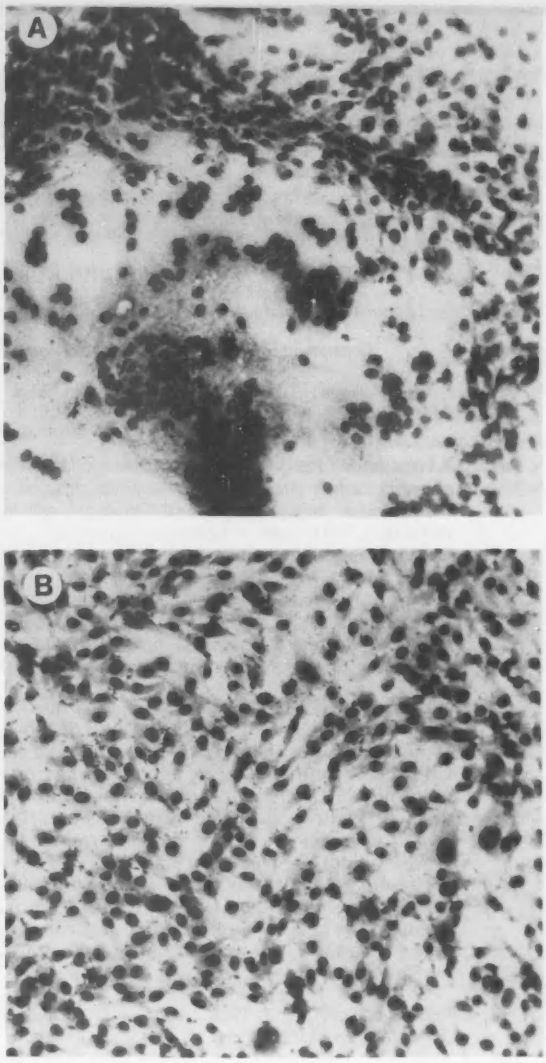

FIG. 1. Photomicrographs of (A) CCO cells infected with F82-34 48 h after inoculation at 26°C and (B) uninfected cells.

The concentrations of CFV and CAV were determined following infection of CCO, BB, and FHM cells after 96 h of incubation at 26°C. The virus yields of both CAV and CFV were similar between the CCO and BB lines (Table 1), indicating that both lines supported viral replication. The FHM line did not show CPE, and virus yields were minimal over the same period.

Electron microscopy — Virus was purified from infected CCO cells by the method described by Hedrick et al. (1983) and examined by electron microscopy to determine the morphology of the virus particles. The virions are double-shelled and icosahedral in shape with an average diameter of 75 nm (Fig. 2).

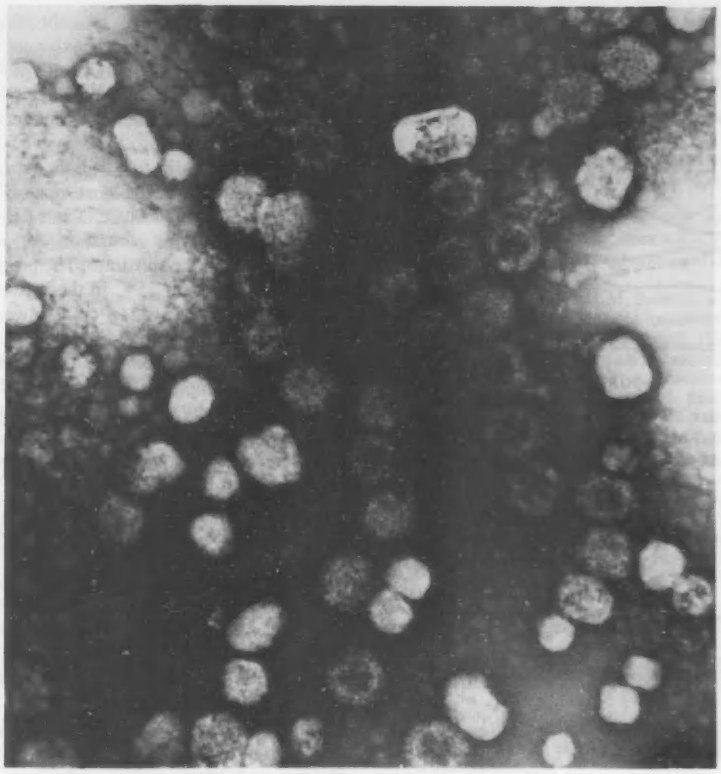

FIG. 2. Electron micrograph of partially purified virions of F82-34. Virions were stained with 1% phosphotungstic acid. The average diameter of the virions was 75 nm.

TABLE 1. Comparison of cell-associated and cell-free virus yields following infection of three cell lines with F82-34 virus. $TCID_{50}$ values represent the average of two replicate flasks infected with F82-34 and incubated for 96 h at 26°C. Virus titers are expressed as $\log_{10}$ $TCID_{50}$/mL.

		$TCID_{50}$/mL	
Cell line	MOI	Cell-free virus	Cell-associated virus
FHM	$1.3 \times 10^{+3}$	3.47	3.69
BB	$4.7 \times 10^{+4}$	4.96	8.13
CCO	$7.8 \times 10^{+4}$	4.23	7.26

The morphology and size is similar to viruses of the family Reoviridae.

Serological properties of F82-34 — Because no virus other than CCV had been isolated from channel catfish, it was first suspected that F82-34 may have been another strain of that virus. Serum neutralization and fluorescent antibody tests, however, failed to show any cross reactions between anti-CCV serum and F82-34 virus.

The neutralization indices of F82-34, CSV, and GSV showed that the three viruses were serologically related (Table 2). The two reoviruses GSV and CSV although related could, however, be distinguished from F82-34 by these methods.

TABLE 2. Serum neutralization indices of three reoviruses isolated from fish using anti-F82-34 virus rabbit serum. Neutralization indices were determined by the difference in titer of virus following a 1-h incubation at room temperature in both normal and anti-F82-34 rabbit serum diluted 1:100 in MEM-5. Virus titers are expressed as $\log_{10}$ $TCID_{50}$/mL.

Virus	Normal serum	Anti-F82-34	Neutralization index
F82-34	6.29	2.83	3.46
GSV	5.38	3.69	1.69
CSV	5.83	3.60	2.23

Effects of temperature — The concentrations of F82-34 released from CCO cells rose initially most rapidly at 30°C (Fig. 3). However, after 96 h of incubation the highest concentrations of cell-free virus were detected at 25°C. Little or no virus replication was found at 40, 35, 20, and 15°C. At the time the experiment was terminated (96 h) a few focal areas of syncytia were forming at 15, 20, and 35°C, indicating that some minimal amount of viral replication was underway. The CCO cells did not survive after 24 h of incubation at 40°C, but continued sampling of the culture fluids indicated that the original virus inoculum was slowly being inactivated over the 96-h period.

The concentrations of CAV at 25°C increased rapidly over 36 h and then stabilized at 5.75 $TCID_{50}$/mL. After 96 h the amounts of CAV and CFV were equivalent (Fig. 3).

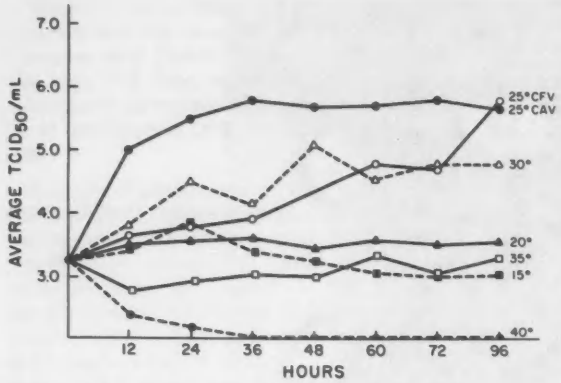

FIG. 3. Effect of temperature on the replication of F82-34 virus in CCO cells. The concentrations of cell-free virus (CFV) were determined at each temperature and the cell-associated virus (CAV) at 25°C only.

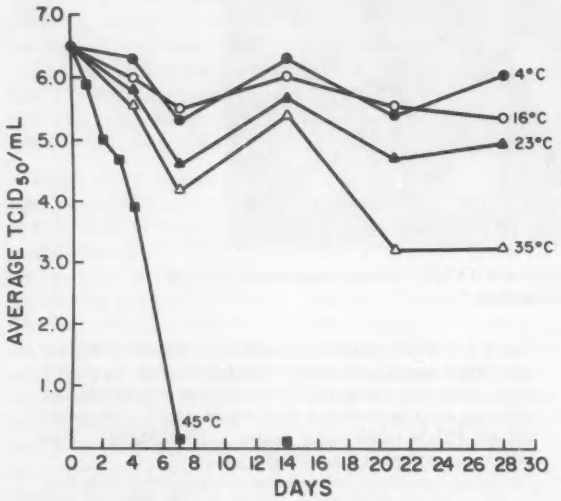

FIG. 4. Concentrations of F82-34 virus detected following incubation at selected temperatures.

F82-34 was quite stable at 4, 16, and 23°C with less than 1.1 $\log_{10}$ $TCID_{50}$ reductions during incubation in MEM-5 over a period of 28 d (Fig. 4). Only 3.3 $\log_{10}$ of virus was lost at 35°C over the same period. All virus infectivity, however, was lost after 7 d at 45°C. The virus could be safely stored at lower temperatures with no losses in infectivity found in samples of F82-34 held in MEM-5 at −20 and −80°C over a 5-mo period.

Discussion

The isolate F82-34 described in this study is the second virus found in channel catfish. It clearly differs from CCV (Plumb 1978) both in particle morphology and serological properties.

Preliminary infectivity trials with F82-34 at 26°C indicate that the virus can replicate for extended periods but has low virulence for channel catfish compared with CCV (unpubl. data). This corresponds with observations in the field where F82-34 has now been isolated from catfish at two different farms, and in neither case was the virus associated with high mortalities. The only observed pathological changes were hyperplasia of the gill lamellae with multifocal fusion and these were regarded as minor and nonspecific. The impact of F82-34 on the health of infected fish under stress (higher or lower temperatures, crowding, low oxygen, etc.) is not known. The source of the virus in fish at both farms is unknown although both draw water from the same open canal system.

The CPE of F82-34 in CCO and BB cells was characterized by massive syncytia formation followed by rounding and detachment from the substrate. The process was completed after 48 h of incubation at 25°C in these two lines but was retarded substantially in CHSE-214 or at lower temperatures (15 and 20°C). A majority of the virus remains cell associated in early stages of infection (Table 1) but approximately 50% is released after CPE is complete (Fig. 3). A very similar CPE and virus yield is induced by GSV in FHM cells (Plumb et al. 1979) and CSV in CHSE-214 cells (Winton et al. 1981). These viruses, however, have phenotypic properties that distinguish them from each other and from CRV.

Although optimal temperatures of replication were not examined with GSV (Plumb et al. 1979), good growth was observed at 30°C, which is near the 25°C optimum for F82-34 but substantially higher than 15°C obtained with CSV (Winton et al. 1981). The three reoviruses can be further distinguished by their cell line specificities and neutralization responses to anti-F82-34 serum (Table 2). Neither GSV nor CSV replicates in CCO or BB cells, which are the preferred lines for F82-34 (Plumb et al. 1979; Winton et al. 1981).

Partially purified virus (F82-34) from infected CCO cells is morphologically similar to viruses in the family Reoviridae (Palmer and Martin 1982). There was no indication of an envelope, and the double capsid was clearly evident. The F82-34 virus is similar in size and morphology to the reoviruses GSV and CSV from fish and another ($13P_2$) from American oysters (*Crassostrea virginica*) described by Meyers and Hirai (1980).

A serological examination of the three reoviruses from fish with anti-F82-34 serum indicated that relationships do exist (Table 2). Although the viruses were isolated from diverse hosts and show several phenotypic differences, they share some common antigens associated with virus neutralization. A comparison of the three viruses by cross-neutralization studies coupled with a characterization of their viral polypeptides and genome segments suggests that the three viruses may represent multiple serotypes of a new group of reoviruses (R. P. Hedrick et al., unpubl. data).

Many of the physical characteristics of GSV and CSV have been determined. The viruses have diameters of 70–75 nm, are stable at pH 3, and are resistant to chloroform and 5-iodo(or fluoro)-2-deoxyuridine (Plumb et al. 1979; Winton et al. 1981). These characteristics combined with acridine orange staining indicated that the viruses were most like the double-stranded RNA viruses grouped in the family Reoviridae. Winton et al. (1983) has further characterized CSV by biochemical examinations of purified virions. Their studies revealed that CSV contained 5 major and 4 minor structural proteins and 11 segments of double-stranded (ds) RNA. Similar structural polypeptides and 11 segments of dsRNA have also been found in F82-34 virus and GSV (R. P. Hedrick et al., unpubl. data).

It is likely that the $13P_2$ virus isolated from oysters (Meyers 1979) will also share similar biochemical properties with CSV, GSV, and F82-34, supporting their placement into a new

taxonomic group within the family Reoviridae. We therefore propose that this new reo-like virus from channel catfish be tentatively designated catfish reovirus (CRV).

Acknowledgments

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Ionic Strength Correction for Extent of Ammonia Ionization in Freshwater

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Messer, J. J., J. Ho, and W. J. Grenney. 1984. Ionic strength correction for extent of ammonia ionization in freshwater. Can. J. Fish. Aquat. Sci. 41: 811–815.

Previously published tabulations of percent ammonia ionization inherently define pH in terms of concentration (molality) rather than the measured entity, hydrogen ion activity. In hard waters this may result in overestimation of the un-ionized NH_3 concentration by 10–20%. The thermodynamic basis for current empirical methods for temperature correction is examined, and a tabulation of NH_3 ionization fraction as a function of pH, temperature, and dissolved solids, together with a corresponding predictive equation, is presented.

Les tableaux publiés dans le passé des pourcentages d'ionisation de l'ammoniac définissent essentiellement le pH en termes de concentration (molalité) plutôt que d'entité mesurée, l'activité de l'ion hydrogène. Dans le cas d'eaux dures, il peut en résulter une surestimation de 10 à 20 % de la concentration de NH_3 non ionisé. Nous examinons le fondement thermodynamique des méthodes empiriques actuelles de correction de la température et décrivons la mise en tableau de la fraction d'ionisation de NH_3 en fonction du pH, de la température et des solides dissous, de même qu'une équation prévisionnelle correspondante.

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The toxicity of ammonia to aquatic life has long been attributed solely to the un-ionized $NH_3(aq)$ species, with its conjugate acid, NH_4^+, showing virtually no toxicity under typical environmental conditions (e.g. Wuhrmann and Woker 1948). Although several workers have questioned the adequacy of the EPA "Red Book" (1976) criterion of 0.016 mg un-ionized NH_3-N/L (=0.02 mg/L NH_3) to protect freshwater life (e.g. Szumski et al. 1982), none of the criticism

has questioned the importance of ionization of NH_3 in determining its environmental toxicity.

Thurston et al. (1979) presented a tabulation of percent un-ionized ammonia as a function of temperature and pH, based on their previously published critical evaluation of the literature (Emerson et al. 1975). In the earlier publication, they stated that the effects of ionic strength at total dissolved solids concentrations below 200–300 mg/L were negligible. This assumption can result in a significant error when their tabulated values are used for hard waters, and we present a corrected tabulation of the fraction of ammonia ionization for a range of pH, temperature, and ionic strength values of environmental interest. A computational formula for use in calculating the fraction of NH_3-N that exists in the toxic, un-ionized form in environmental fate and effects models is also given.

Theoretical Basis

Emerson et al. (1975) based their equilibrium calculations on the original work of Bates and Pinching (1949), who presented the following thermodynamic equation for the acid dissociation of ammonium ion:

$$(1) \quad K_a^0 = \frac{(M_{H^+})(f_{H^+})(M_{NH_3})(f_{NH_3})}{(M_{NH_4^+})(f_{NH_4^+})(a_w)}$$

where $K_a^0 = 10^{-9.245}$, M represents the molality of the subscripted species, f represents the corresponding activity coefficient, and a_w is the activity of the aqueous solvent. Bates and Pinching (1949) subsequently employed electromotive force measurements using a hydrogen–silver chloride cell to determine the acidity function, pwH ($= -\log (f_{H^+} f_{Cl^-} M_{H^+})$) which, when substituted into their equation (4), yields equation (1) above.

Accounting for NH_3 losses from the system, solubility of AgCl in the cell, and several other factors, Bates and Pinching (1949) tabulated values for $-\log K_a$ in their table 3, which they also found showed a good fit to the equation

$$(2) \quad -\log K_{a,T} = \frac{A}{T_K} + B$$

in which the numerical values of A and B were 2706 and 0.139, respectively. This equation is, in fact, an empirical fit to the van't Hoff relationship

$$(3) \quad -\ln K_{a,T} = \frac{\Delta G}{RT_K}$$

into which the thermodynamic relationship

$$(4) \quad \Delta G = \Delta H - T_K \Delta S$$

is substituted to give

$$-\ln K_{a,T} = \frac{\Delta H}{RT_K} - \frac{\Delta S}{R}$$

where ΔH represents the free enthalpy of formation, ΔS is the entropy of formation, R is the thermodynamic gas constant (8.314 J/mol-°K), and T_K is the Kelvin temperature. It can be seen that A in equation (2) should be virtually equal to $\Delta H/(2.303R)$, and B to $-\Delta S/(2.303R)$. Emerson et al. (1975) confirmed the fit of equation (2) using statistical computing techniques not available to Bates and Pinching (1949).

The van't Hoff relationship would be expected to adequately describe the temperature dependence of K_a, provided that ΔH is not strongly dependent on temperature over the range of interest. This will occur if there is not a significant change in the thermodynamic heat capacities of the reactants or the products in the reaction in question (e.g. Stumm and Morgan 1981, p. 68–72). Exactly such independence of ΔH with temperature over the range 5–145°C has been demonstrated by Olofsson (1975); thus, the use of equation (2) to describe the effects of temperature on ammonia ionization, as adopted by Emerson et al. (1975), appears to have a sound thermodynamic basis.

The dependence of the ionized fraction on ionic strength can be seen by dissecting equation (1) into a term describing ion concentrations and one describing ion activity coefficients, as has been done by Whitfield (1974) for seawater activity corrections:

$$(5) \quad K_{a,T} = \frac{(M_{NH_3})(M_{H^+})}{M_{NH_4^+}} \times \frac{(f_{NH_3})(f_{H^+})}{(f_{NH_4^+})(a_w)}.$$

At low ionic strength, f_{NH_3} and a_w are virtually equal to unity (Bates and Pinching 1949) and, following Whitfield (1974), at low ionic strength the single ion activity coefficients for H^+ and NH_4^+ are virtually identical and thus cancel out. The resulting equation, in which the right-hand factor becomes unity, now indicates a relationship between the ionized fraction of the analytical concentration of NH_3-N ($M_{NH_3}/M_{NH_4^+}$) and hydrogen ion *concentration*. However, the pH value measured by potentiometric methods, reported in the literature, and invariably used for fate and effects modeling, represents the negative logarithm of the hydrogen ion *activity* (APHA 1981):

$$(6) \quad \text{pH} = -\log (M_{H^+} f_{H^+}) = -\log (a_{H^+}).$$

Therefore, equation (5) could more usefully be written

$$(7) \quad K_{a,T}^c = (f_{NH_4^+})(K_a) = \frac{M_{NH_3}}{M_{NH_4^+}} a_{H^+}.$$

The conditional stability constant, K_a^c, can clearly be seen to depend on $f_{NH_4^+}$.

Thermodynamically rigorous means exist for calculating activity coefficients for specific ions in well-defined electrolyte solutions such as seawater (Whitfield 1974) and should be used for freshwaters for which major ion analyses are available. In cases for which these data are either unavailable or highly variable, the Guntelberg approximation of the Debye–Huckel formula can be used to calculate $f_{NH_4^+}$, based on the ionic strength, I, under conditions in which $I < 0.1\,M$ (Stumm and Morgan 1981, p. 134–137):

$$(8) \quad \log f_{NH_4^+} = \frac{-A' I^{1/2}}{1 + I^{1/2}}$$

where A' is a coefficient depending on the dielectric constant (E) and the Celsius temperature (T_C) of the water, expressed as

$$(9) \quad A' = 1.82483 \times 10^6 \times [E \times (T_C + 273.16)]^{-1.5}.$$

The dielectric constant was reported by Truesdell and Jones (1974) to be

$$(10) \quad E = 87.74 - 0.4008 \times T_C + 9.398 \times 10^{-4} \times T_C^2 - 1.41 \times 10^{-6} \times T_C^3.$$

If insufficient data for an exact calculation exist, the ionic

TABLE 1. Percent un-ionized ammonia, $NH_3(aq)$, in freshwater (TDS = 0–1600 mg/L) as a function of total dissolved solids, temperature, and pH. Ionic strength was calculated using equation (12).

		TDS (mg/L)				
T (°C)	pH	0	400	800	1200	1600
0	6.0	0.00824	0.00743	0.00716	0.00697	0.00682
	6.5	0.0260	0.0235	0.0226	0.0220	0.0216
	7.0	0.0823	0.0743	0.0716	0.0697	0.0682
	7.5	0.260	0.235	0.226	0.220	0.215
	8.0	0.817	0.738	0.711	0.692	0.678
	8.5	2.54	2.30	2.22	2.16	2.11
	9.0	7.61	6.92	6.68	6.52	6.39
	9.5	20.67	19.04	18.47	18.07	17.70
	10.0	45.18	42.64	41.73	41.08	40.60
10	6.0	0.0186	0.0167	0.0161	0.0157	0.0153
	6.5	0.0587	0.0529	0.0509	0.0496	0.0485
	7.0	0.185	0.167	0.161	0.157	0.153
	7.5	0.584	0.526	0.507	0.493	0.483
	8.0	1.82	1.65	1.59	1.54	1.51
	8.5	5.55	5.03	4.85	4.72	4.63
	9.0	15.66	14.33	13.87	13.55	13.30
	9.5	37.00	34.60	33.75	33.14	32.66
	10.0	65.00	62.59	61.70	61.05	60.53
15	6.0	0.0273	0.0246	0.0237	0.0230	0.0225
	6.5	0.0863	0.0777	0.0747	0.0727	0.0711
	7.0	0.0272	0.0245	0.0236	0.0230	0.0225
	7.5	0.856	0.771	0.742	0.722	0.707
	8.0	2.66	2.40	2.31	2.25	2.20
	8.5	7.95	7.21	6.96	6.78	6.65
	9.0	21.45	19.73	19.13	18.71	18.37
	9.5	46.33	43.73	42.79	42.12	41.58
	10.0	71.19	71.08	70.28	69.71	69.24
20	6.0	0.0396	0.0356	0.0343	0.0333	0.0326
	6.5	0.125	0.113	0.108	0.105	0.103
	7.0	0.395	0.355	0.342	0.332	0.325
	7.5	1.24	1.11	1.07	1.04	1.02
	8.0	3.81	3.44	3.31	3.23	3.16
	8.5	11.13	10.12	9.78	9.54	9.35
	9.0	28.37	26.26	25.52	25.00	24.59
	9.5	55.60	52.97	52.01	51.32	50.76
	10.0	79.84	78.08	77.41	76.92	76.53
25	6.0	0.0567	0.0510	0.0490	0.0477	0.0466
	6.5	0.179	0.161	0.155	0.151	0.147
	7.0	0.564	0.507	0.488	0.475	0.464
	7.5	1.76	1.57	1.53	1.49	1.45
	8.0	5.37	4.85	4.67	4.55	4.46
	8.5	15.21	13.88	13.43	13.10	12.85
	9.0	36.20	33.77	32.90	32.29	31.80
	9.5	64.21	61.72	60.80	60.13	59.59
	10.0	85.01	83.60	83.06	82.66	82.34
30	6.0	0.0803	0.0721	0.0693	0.0674	0.0659
	6.5	0.253	0.228	0.219	0.213	0.208
	7.0	0.797	0.716	0.689	0.670	0.655
	7.5	2.48	2.23	2.15	2.09	2.04
	8.0	7.44	6.73	6.48	6.31	6.18
	8.5	20.26	18.57	17.98	17.57	17.24
	9.0	44.55	41.90	40.95	40.26	39.72
	9.5	71.76	69.51	68.68	68.07	67.57
	10.0	88.93	87.82	87.40	87.08	86.82

TABLE 2. Un-ionized NH_3–N in 14 water samples from the Jordan River, Utah (1979–80), based on salinity correction using equations (13) and (11) and on the uncorrected U.S. EPA compilation of Thurston et al. (1979). All values in mg/L except pH, temperature (°C), and alkalinity (mg/L as $CaCO_3$). Data are from USGS (1981).

Sample No.	pH	Water temperature	Ca^{2+}	Mg^{2+}	Alkalinity	TDS	Total NH_3–N	Un-ionized NH_3–N	
								Uncorrected	Corrected
1	8.0	21.0	59	47	180	763	0.05	0.002	0.002
2	7.7	19.5	102	52	240	1030	1.2	0.023	0.019
3	7.3	12.5	60	29	150	537	0.70	0.003	0.003
4	7.9	7.5	130	55	270	1130	3.4	0.041	0.035
5	7.5	17.0	110	53	260	1060	1.6	0.016	0.014
6	7.7	12.5	130	56	260	1140	2.0	0.022	0.019
7	7.6	17.0	91	56	230	1010	1.2	0.015	0.013
8	8.0	14.0	66	27	170	512	0.43	0.011	0.010
9	7.9	14.0	100	57	230	1070	1.4	0.028	0.024
10	7.8	9.0	120	52	240	1060	2.7	0.029	0.025
11	8.2	6.5	92	55	220	994	1.1	0.024	0.021
12	8.0	8.5	87	55	230	981	1.2	0.020	0.017
13	8.0	13.5	63	36	205	664	0.56	0.013	0.012
14	8.0	20.0	63	41	180	731	0.05	0.002	0.002

strength can be approximated using either

$$(11)\quad I = 4 \times ([Ca^{2+}] + [Mg^{2+}]) - 1/2[Alk]$$

or

$$(12)\quad I = 2.5 \times 10^{-5} \times TDS$$

where $[Ca^{2+}]$ = molar concentration of Ca^{2+} (moles per litre), $[Mg^{2+}]$ = molar concentration of Mg^{2+} (moles per litre), [Alk] = alkalinity of the water (equivalents per litre), and TDS = total dissolved solids of the water (milligrams per litre). It should be noted, however, that use of equation (12) could result in errors of as much as twofold in waters of substantially different major ion composition (e.g. calcium sulfate versus sodium chloride). The un-ionized fraction for ammonia now can be expressed in the convenient form

$$(13)\quad \frac{(M_{NH_3})}{(M_{NH_3}) + (M_{NH_4^+})} = \frac{1}{1 + 10^{(pK_{a,T} - pH - s)}}$$

where s represents the salinity correction term corresponding to the right-hand side of equation (8) and pH is the potentiometrically determined negative logarithm of the hydrogen ion activity. From equation (13) it can be seen that the ionic strength correction is co-equal in importance with hydrogen ion activity in determining the un-ionized fraction of ammonia in aquatic systems.

Results and Discussion

A tabulation of NH_3-N ionization fractions at various pH, TDS, and temperature values, expressed as percent un-ionized NH_3, is presented in Table 1. For values other than those tabulated, either equation (13) can be used directly, or values in previous tabulations can be multiplied by $f_{NH_4^+}$ as calculated using equation (8). Although the Davies approximation has been suggested by Stumm and Morgan (1981) for use at ionic strengths between 0.1 and 0.5 M, it is advisable to use the more rigorous activity coefficient calculations of Whitfield (1974) for estuarine and seawater ($I = 0.7\ M$) systems.

To show the effects of the resulting corrections for ionic strength, water quality data based on 14 samples from the Jordan River, Utah, were used to calculate un-ionized NH_3-N concentrations using equations (13) and (11). Virtually identical values were obtained using equation (12). The results are compared (Table 2) with the corresponding values from the compilation of Thurston et al. (1979) for waters of zero salinity. The corresponding EPA values are 10–19% higher than those corrected for ionic strength, with the lower errors corresponding to the lower TDS values (=500–550 mg/L). Similar differences are likely when applying the tabulations of Whitfield (1974) and Bower and Bidwell (1978).

The errors likely to result from using ionization fractions that are uncorrected for salinity in most freshwaters are small (15–20%) and in the range of error expected in pH measurements to result from diurnal pH variations or the use of less sensitive or improperly calibrated field instruments. It is also possible that the 0.1 application (i.e. "safety") factor used to calculate the criterion value is sufficiently conservative to ensure that a discrepancy of 10% between the actual and apparent un-ionized ammonia concentrations would not elicit an observable acute response in a field population of fish. However, in toxicological studies used as a basis for establishing water quality criteria and standards, it is essential that the correct un-ionized ammonia concentration be known.

It is significant that, despite the fact that random variation in field pH measurements may result in errors in the ionization fraction similar in magnitude to the ionic strength correction, failure to correct for data set in ionic strength will bias the *mean* value of un-ionized ammonia for a large set of field data. The latter problem is particularly disturbing if the incorrect values are used in a regulatory setting. Wastewater discharge permits are usually predicated on the number (or fraction) of times that water quality standards are exceeded. For the data in Table 2, when corrected for ionic strength, only 4 of the 14 samples (29%) exceed the 0.02 mg/L un-ionized NH_3-N Utah Water Quality Standard instead of 6 (43%) as determined using the tabulations of Thurston et al. (1979). Such an increase of 50% in the number of times standards are exceeded can lead to construction of advanced nitrogen removal facilities costing many millions of dollars. Such construction may not be justified if the safety factor inherent in the 0.02 mg/L un-ionized NH_3-N standard is already adequate to protect fisheries in the receiving stream reach.

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A Method for the Analysis of Total Nitrogen in Natural Waters[1]

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Kalff, J., and E. Bentzen. 1984. A method for the analysis of total nitrogen in natural waters. Can. J. Fish. Aquat. Sci. 41: 815–819.

This method for analyzing total nitrogen (TN) in freshwaters is based on the persulfate oxidation of nitrogen to nitrate, followed by the analysis of this nitrate by a modified version of the sodium salicylate method. The method is simpler than other reported techniques for TN in oligotrophic and mesotrophic waters and requires equipment readily available in most laboratories. The method is linear to 1000 μg N/L, with the range extendable by changing the sample size. The variability is lowest (coefficient of variation 6.6%) between 100 and 1000 μg N/L. We have successfully used the method for the determination of TN, as well as dissolved nitrogen (DN) on filtered samples and nitrate on nonoxidized samples.

La méthode décrite dans l'article qui suit pour analyser l'azote totale (TN) dans les eaux douces est fondée sur l'oxydation au persulfate d'azote en nitrate, suivie du dosage de ce dernier suivant une version modifiée de la méthode du salicylate de sodium. Cette méthode est plus simple que celle déjà décrite pour le dosage de TN dans des eaux oligotrophes et mésotrophes, avec l'équipement dont dispose la plupart des laboratoires. La méthode est linéaire jusqu'à 1000 μg N/L, cette gamme pouvant être élargie en modifiant le volume de l'échantillon. La variabilité est la plus faible (coefficient de variation de 6,6 %) entre 100 et 1000 μg N/L. Nous avons utilisé ce procédé avec succès pour la détermination de TN ainsi que celle de l'oxygène dissous (DN) sur des échantillons filtrés et du nitrate dans des échantillons non oxydés.

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Measurement of total nitrogen (TN) in freshwater has until recently involved separate analysis of a number of nitrogen fractions and their subsequent summation. This is both time consuming and subject to cumulative analytical errors. Two techniques proposed to circumvent these problems make a single measure of total nitrogen by oxidizing, under pressure, all nitrogen forms in the water sample to nitrate, followed by reduction of the oxidation product and its subsequent measurement. In one, the oxidation product is reduced to ammonium with an alloy (Raveh and Avnimelech 1979) and the ammonia measured by a standard method. Although appealingly simple, the Devarda alloy used contains sufficient nitrogen to make it unsuitable for all but eutrophic waters. In the second method, the oxidation product is reduced to nitrite in a cadmium or cadmium–copper column prior to spectrophotometric analysis (Koroleff 1976; Nydahl 1978; Valderrama 1981). The same technique has been used for the analysis of dissolved nitrogen (DN) (Solorzano and Sharp 1980). The reduction procedure was initially developed for the measurement of nitrate plus nitrite (Morris and Riley 1963;

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American Public Health Association 1975) and thus did not originally include a persulfate oxidation step. The reduction columns for nitrate plus nitrite appear to work well in experienced hands but require careful preparation, regular verification of their reduction efficiency, which changes with use, periodic rejuvenation, and the determination of optimal flow for each column. In addition, we have encountered considerable problems with column clogging and consequent changes in reduction efficiency in passing the persulfate oxidation product through the columns (H. Evans and J. Kalff, unpubl. data).

In response to the above problems, we developed a method that combines persulfate oxidation with subsequent analysis of the nitrate produced with the salicylate method. The oxidation uses solid potassium persulfate as opposed to the commonly used liquid preparation of oxidant (Solorzano and Sharp 1980), eliminating the possibility of changes in oxidation potential with aging of the solution. The salicylate method was developed in Germany (Müller and Widermann 1955) and has been used in South Africa (National Institute for Water Research 1974) and Israel (Scheiner 1974) for the analysis of nitrate in fresh and wastewaters. In it, sodium salicylate reacts with nitrate ions in a sulfuric acid medium to form nitrosalicylic acid, which turns yellow under alkaline conditions. The combined method is simple, reproducible, and economical, requiring only standard apparatus, a spectrophotometer, a pressure cooker or autoclave, and hotplates and is useful for routine measurement of nitrogen in the field. The same method can be used to determine nitrate, TN, and DN.

Materials and Methods

An amount of 240 g of NaOH (6 mol/L) was dissolved in deionized–distilled water, diluted to 1 L, and stored in a polypropylene bottle. The solution is stable for months. Low-nitrogen analytical grade potassium persulfate in powder form was used as the oxidizing reagent. Lowest blanks were obtained with the persulfate from BDH; recrystallization is not necessary. Concentrated (2 mol/L) H_2SO_4 (11.1 mL) was added to 89.0 mL of distilled water. Ten grams of 1% sodium salicylate ($C_6H_4(OH)$-$COONa$) was dissolved in distilled water, diluted to 1 L, and stored in a dark glass bottle at 4°C. The solution is stable for 2 mo. An amount of 50 g of sodium potassium tartrate (Rochelle's salt) ($KOCO(CHOH)_2COONa \cdot 4H_2O$) was dissolved in water and diluted to about 550 mL. While swirling, 400 g of NaOH, 50 g at a time, was slowly and carefully added, dissolving all the pellets before each new addition, cooled, diluted to 1 L, mixed well, and stored in a polypropylene bottle. The solution is stable for months. The following solutions were prepared by dissolving in water and diluting to 1 L: standard KNO_3 (0.5000 g N/L), 3.6092 g; standard urea (NH_4CONH_2) (0.5000 g N/L), 1.1079 g; standard ammonia (NH_4NO_3 (0.5000 g N/L), 1.4287 g. Working standards (diluted) for analysis were prepared from the stock solutions over the range of 100–1000 μg N/L. All reagents were prepared with only analytical grade chemicals and high-quality deionized–distilled water. All glassware must be well cleaned and rinsed copiously with high-quality water and dried in a dust-free environment.

An amount of 35 mL (or an appropriate volume) of the well-mixed water sample was measured into 60-mL culture tubes with nonlined polypropylene caps. An amount of 0.14 g of potassium persulfate (we had a scoop made to measure out the persulfate) and 0.70 mL of 6 mol NaOH/L were added. The tubes were capped and autoclaved at 103 kPa (15 psi) for 40 min and cooled. An amount of 5.00 mL of the oxidized sample was subsampled into a clean 50-mL Erlenmeyer flask. An amount of 0.090 mL of H_2SO_4 (2 mol/L) was added followed by 2.0 mL of 1% sodium salicylate. The flasks were covered with watchglasses and the samples evaporated, but not boiled, to complete dryness at about 98°C on a hotplate (an oven or a water bath can be used). Once dry, they were removed immediatley and 1.0 mL of concentrated H_2SO_4 was added, swirling the acid over the dried sample and allowing it to dissolve for at least 10 min. About 20 mL of distilled water was added and mixed well. The water added will clear any remaining turbidity. Five millilitres of Rochelle salt was added and mixed well and diluted quantitatively to 55.0 mL in precalibrated 50-mL Erlenmeyers flasks. Alternatively, an exact volume of water may be added to the acidified samples with a reliable dispenser (i.e. 30–40 mL or more), followed by the Rochelle salt, thus eliminating the need to dilute to a mark. The Erlenmeyer flasks were sealed with parafilm, mixed thoroughly and read in 10-cm glass cuvettes on a spectrophotometer at 420 nm. Blanks were prepared by measuring 35 mL of high-quality distilled or deionized water blanks and 35-mL standards into culture tubes and following through the entire procedure. The mean blank absorbance was subtracted from the standard and sample absorbances and a standard curve established. With subsequent analyses, only one concentration of standard was sufficient for verification purposes.

The sample concentration was calculated as follows:

$$\text{Sample concn.} = \frac{A_x}{\text{slope}} \ (\mu g/L)$$

where the slope is derived from the standard curve and A_x is the corrected sample absorbance.

The salicylate method is equally suitable for nitrate and DN. For nitrate, the sample water was gently filtered (<28 kPa) through prewashed (by soaking in distilled water) 0.45-μm membrane filters and the first 50 mL of filtrate discarded. An amount of 5.0 mL of filtrate was collected into a 50-mL Erlenmeyer flask. The procedure for TN, as above, was followed beginning with the addition of salicylate, but not the 2 mol H_2SO_4/L. Make a correction for natural color in dystrophic waters. For DN, the sample was filtered as for nitrate, but 35.0 mL of the filtrate was collected and the procedure for TN followed.

Results and Discussion

Control of the pH at the different steps is essential. Complete oxidation of the reduced forms of nitrogen with persulfate requires an alkaline medium. Solorzano and Sharp (1980) recommend a pH range for this of 12.6–13.2, while Valderrama (1981) considered a pH of 9.7 as sufficient. Before the oxidation we added 0.7 mL of 6 mol NaOH/L to the 35-mL sample, yielding a postoxidation pH of 12.3. This addition was sufficient to offset the sulfuric acid produced during the persulfate reduction but also sufficiently small in volume not to dilute the original sample significantly. The addition of 2 mol H_2SO_4/L prior to the hotplate drying of the oxidized samples (pH 2.0–3.0) produces a suitable noncrystalline solid upon drying. For unknown reasons, drying with an initial pH less than 1.5 inhibits the reaction with salicylate, yielding final solutions with reduced color. Conversely, basic samples upon drying develop a crystalline residue that causes turbidity in subsequent steps. As the formation of nitrosalicylic acid requires strongly

TABLE 1. Mean observed and expected absorbance values for a range of nitrogen standards (urea, tris, ammonium sulfate, and potassium nitrate in order of decreasing frequency) using the persulfate–salicylate method for TN. Absorbances were corrected for the blank and read in a 10-cm glass cuvette at 420 nm. The 95% confidence interval (CI) estimates for each standard are given, and all absorbance values are significantly different from each other ($P < 0.05$), even where the 95% CI is large. Analysis of variance and regression statistics: intercept = 0.0001, slope (bi) = 0.0006881, % CV of regression = 6.18, correlation coefficient (R^2) = 0.992, F value ($n = 57$) = 6890, means square error = 0.0003684.

Standard concentration (μg/L)	n	Mean observed absorbance	SD	% CV	Regression-derived absorbance	Regression-calculated concentration	95% CI
25	3	0.014	0.0017	12.1	0.017	20.4	15.9, 24.9
50	6	0.026	0.0067	25.8	0.034	37.8	27.6, 48.0
100	3	0.068	0.0100	14.7	0.069	98.8	62.7, 135
200	3	0.156	0.0084	5.4	0.138	227	197, 257
300	3	0.194	0.018	9.3	0.206	282	217, 347
500	21	0.349	0.019	5.4	0.344	507	495, 520
600	3	0.418	0.009	2.2	0.413	608	576, 641
706	4	0.486	0.030	6.2	0.486	706	637, 775
800	6	0.547	0.030	5.5	0.551	795	794, 841
1000	5	0.682	0.029	4.2	0.688	991	939, 1043
Mean							
25–1000				9.1			
100–1000				6.6			

acidic conditions, 1 mL of concentrated sulfuric acid was added to the dried samples. The yellow color to be measured spectrophotometrically is produced instantly when the nitrosalicylic acid is made alkaline (pH 12) upon the addition of the highly alkaline Rochelle salt.

We did not specifically investigate the completeness of persulfate oxidation, a topic studied by Nydahl (1978) who investigated 47 nitrogen-containing compounds. His work allows the conclusion that the most common nitrogenous compounds found in natural water samples are oxidized (92–100%) with persulfate. Compounds such as urea, ammonia, and nitrate were fully oxidized. Only organic molecules with double bonds between N atoms oxidize much more poorly. We confirmed that the persulfate–salicylate method totally oxidized at least 1000 μg N/L ammonia, tris, and urea, yielding the same color development as standards containing the equivalent concentration of nitrogen as potassium nitrate.

The alternative persulfate–cadmium reduction method uses a liquid persulfate. As there is some question as to its stability in solution (Solorzano and Sharp 1980) and as it does not dissolve easily, we used solid persulfate at 0.4% (w/v) followed by a small volume of concentrated base. This has the added advantage of minimal dilution and thereby a reduced error of the original sample while at the same time simplifying the initial subsampling of the water sample. As high precision in measuring the volume to be digested is not critical, a graduated cylinder suffices. Preparation and verification of a liquid oxidizing reagent is also eliminated.

The universal problem with nitrogen assays, independent of technique chosen, is acceptable reproducibility and adequate sensitivity. The overall coefficient of variation (% CV) for urea, ammonium sulfate, tris, and potassium nitrate standards was 9.1% over the range of 25–1000 μg N/L and 6.6% between 100 and 1000 μg N/L, the range encountered in our lakes, which range from very oligotrophic to highly eutrophic (Table 1). For natural water samples, our technique had an overall mean CV of 13.7% (Table 2). The precision obtained for both standards and water samples seems reasonable but, unfortunately, no comparisons of precision are possible with other published TN techniques, as these lack clear-cut statistics. D'Elia et al. (1977) incorrectly based their %CV values on the standard deviation of means grouped into specified concentration ranges, with the means used being averages of pairs of observations. Solorzano and Sharp (1980) only gave information on performance statistics for DN, while Valderrama (1981) based his estimate of precision on 14 replicates of a single concentration of a urea standard.

Sensitivity with the persulfate–salicylate method is good. Although we measured standards down to 25 μg N/L, the 6.6% CV for standards above 100 μg N/L suggests that the most reliable estimates will be for samples with a TN concentration also greater than 100 μg N/L. It should be possible to increase the sensitivity by either increasing the volume of digested sample to be evaporated, thereby increasing the number of nitrogen molecules reacting with salicylate (Table 3), or by decreasing the final dilution volume, since it is necessary only to have sufficient volume to fill the 10-cm standard cuvettes (26 mL). The addition of the water and Rochelle salt with a reliable dispenser allows a final volume of less than the 55 mL recommended earlier. Similarly, the range could be extended beyond 1000 μg N/L by decreasing the volume evaporated. However, only a single test of doubling the volume evaporated was done with a natural sample (Table 3). The doubling raised the absorbance twofold but without a significant increase in the blank.

The blank absorbances of 0.025–0.05 in a 10-cm cell with the salicylate method are lower than those obtained by other techniques (Table 4). Solorzano and Sharp (1980) cited blanks of 0.09–0.16 in a 1-cm cell (for DN), D'Elia et al. (1977) measured a range of 0.02–0.05 in a 1-cm cell, and Valderrama (1981) obtained one of 0.230 in a 5-cm cell. The latter used a single persulfate digestion for TN and total phosphorus (TP). The high N content of low P persulfate (and vice versa) probably is responsible for the high blank and argues against a combined digestion for both compounds. Our best blanks were obtained

TABLE 2. Mean concentration of TN from nine southern Quebec lakes, based on triplicate analysis, using the persulfate–salicylate method. Nitrate samples were determined on 0.45-μm (Millipore) filtered water, in triplicate, using the salicylate method.

Lake[a]	TN mean concentration (μg/L)	SD	% CV	NO_3 mean concentration (μg/L)	SD	% % CV
Brome	252	15.9	6.3	24.8	9.9	39.9
Waterloo	880	49.4	5.6	78.7	5.9	7.6
Argent	270	73.6	27.2	39.1	3.4	8.8
Brompton	277	41.0	14.8	133	6.2	4.7
Orford	216	83.4	38.5	20.3	9.1	44.7
Massawippi	391	43.1	11.0	206	35.2	17.1
Magog	395	37.1	9.4	28.2	3.0	10.5
Bowker	254	50.3	19.8	130	3.4	2.6
Memphremagog						
Pender	376	61.0	16.2	206	6.4	3.1
	410	0.9	0.02	180	6.8	3.8
Central	401	20.4	5.2	208	21.8	10.5
	321	46.5	18.1	153	17.4	11.4
Border	365	18.9	5.1	206	16.0	7.8
North	305	55.2	14.5	144	6.8	4.7

[a]First eight lakes, data from 1982. Memphremagog data from its four basins, June 1983.

TABLE 3. Comparison of absorbances obtained upon the evaporation of both a 5.0- and 10.0-mL sample and associated blanks for Lake Memphremagog. The two blanks do not differ significantly ($P > 0.2$).

	Absorbance units	
	5.0-mL sample	10.0-mL sample
Blank		
$\bar{x}$	0.037	0.041
SD	0.0059	0.0031
Sample		
$\bar{x}$	0.182	0.368
SD	0.024	0.015

TABLE 4. Means for typical blank values from separate experiments, in absorbance units, for both persulfate-digested and nondigested water samples. Each mean is based on three determinations. The detection limit is calculated as 2 times the standard deviation of the blank (Skoog and West 1980).

Persulfate-digested distilled water		Nondigested distilled water	
$\bar{x}$	SD	$\bar{x}$	SD
0.043	0.0023	0.024	0.0006
0.035	0.0021	0.023	0.0044
0.021	0.0021	0.024	0.0006

using a powdered low-nitrogen persulfate. Although our blank detection limit was about 7 μg N/L on three randomly selected dates, the higher variance associated with natural water samples (Table 2) shows that even an excellent blank detection limit is an inappropriate measure for TN detection in nature. With a very similar variance for TN and nitrate samples and with the latter measured at 20 μg N/L, the sample detection limit for TN must also be about 20 μg N/L. A much higher blank detection limit of 32 μg N/L on a fourth date (data not presented) emphasizes the need for clear glassware and high-quality deionized and distilled water.

Choice of any technique is governed by its simplicity, economy, sensitivity, and precision. The persulfate–salicylate method compares favorably in all respects. Precision is good and the blanks are low and reproducible. Although the method involves a number of steps, the overall procedure is simple and, in our opinion, less cumbersome than the cadmium–copper reduction technique for DN only. Timing of reagent addition is not critical, and the yellow color produced is stable for at least 22 h. The same method allows the measurement of dissolved nitrogen and nitrate.

These advantages are tempered by a limitation, the existence of which is all too rarely acknowledged in technique papers. This limitation, shared with all other persulfate digestion techniques for TN, is the possibility of differing digestion efficiences in different water samples (see Nydahl 1978). Although only specifically addressed in the present study for ammonia, tris, and urea, the Nydahl (1978) work and that of Solorzano and Sharp (1980) and/or our own indirect evidence suggest that it is not a major problem. If it were, a less significant correlation could have been expected in our nine study lakes (J. Kalff and E. Bentzen, unpubl. data) between TN and chlorophyll *a* (Chl *a*) than between TP and Chl *a* as a result of an unreliable estimate of TN. However, the correlation coefficient between TN and Chl *a* was high (0.73, $n = 29$) and as highly significant ($P < 0.0001$) as for TP and Chl *a* ($r = 0.93$, $n = 29$, $P < 0.0001$). This suggests no major changes and differences in oxidation efficiency within and between lakes.

The most time-consuming step in the persulfate–salicylate method is the drying of the 5-mL subsamples. Although batches could be dried more uniformly in a water bath, we selected for more rapid speed with hotplates set at about 98°C and taking 45 min to 1 h to dry the samples. The lack of precise temperature

control and temperature differences over different portions of the heating surfaces results in somewhat unequal drying speed and variation in the time that samples are exposed to a relatively high temperature following evaporation. As sodium salicylate degrades at high temperatures, samples must be removed immediately following evaporation. It is these temperature differences that we suspect are responsible for most of the still modest % CV obtained.

Before the technique is applied to marine waters the observation that chloride might interfere with the nitrosalicylate formation (Scheiner 1974) should be explored.

We use the technique routinely for TN, DN, and nitrate in our lake studies and are able to process 40 TN samples in about 3 h, after the persulfate oxidized samples have cooled.

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Seasonal and Phenotypic Variations in Plasma Protein Antifreeze Levels in a Population of Marine Fish, Sea Raven (*Hemitripterus americanus*)[1]

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The annual cycle of plasma antifreeze polypeptide levels and Na^+ and Cl^- concentrations were measured in sea raven (*Hemitripterus americanus*) from a New Brunswick population. Both Na^+ and Cl^- concentrations showed a distinct seasonal cycle, with the highest concentration of both ions occurring during the winter months. Mean plasma antifreeze levels also showed a significant seasonal cycle, with the highest values occurring during winter. Two features of the annual plasma antifreeze cycle differed considerably from the annual cycles observed in other temperate water fish: antifreeze polypeptides were present throughout the year and, during the winter, 40–50% of the fish had antifreeze levels that were the same as those observed during the summer. Thus, our results modify the generally accepted view that the presence of plasma antifreeze proteins correlates with the seasonal period of low water temperatures and that only fish inhabiting polar oceans maintain high levels of antifreeze peptides in their blood all year round.

[1]Marine Sciences and Research Laboratory Contribution No. 538.

Le cycle annuel des niveaux de polypeptides antigel du plasma et des concentrations de Na^+ et Cl^- a été mesuré chez des hémitriptères atlantiques (*Hemitripterus americanus*) d'une population du Nouveau-Brunswick. Les concentrations de Na^+ et Cl^- montrent toutes deux un cycle saisonnier distinct, les concentrations maximales des deux ions se trouvant dans les mois d'hiver. Les niveaux moyens d'antigel plasmique accusent également un cycle saisonnier prononcé, les valeurs maximales se rencontrant en hiver. Deux caractéristiques du cycle antigel plasmique annuel diffèrent considérablement des cycles annuels observés chez d'autres espèces de poissons des eaux tempérées : les polypeptides antigel sont présents tout au long de l'année et, en hiver, 40–50 % des poissons ont des niveaux d'antigel identiques à ceux observés en été. Nos résultats viennent donc modifier le point de vue généralement accepté que la présence de protéines antigel plasmiques est liée à la saison de basses températures de l'eau et que seuls les poissons habitant les eaux polaires maintiennent à l'année longue des niveaux élevés de peptides antigel dans le sang.

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The blood plasma freezing point of most teleost fishes that inhabit temperate marine waters is approximately −0.6 to −0.8°C (Holmes and Donaldson 1969). Although these fish can be supercooled to temperatures of −2 to −3°C in the absence of ice, if ice is added to the water, ice crystals appear to penetrate the epithelium and immediately cause the fish to freeze and die (Scholander et al. 1957). Clearly, such fish would be incapable of surviving in polar and subpolar oceans, which are characterized both by subzero water temperatures (−1.4 to −2°C) and the presence of ice. Extensive investigations over the past decade have established that many teleost fishes inhabiting icy marine waters differ from their more temperate counterparts in that they possess peptide or glycopeptide antifreezes. These compounds lower the freezing point of the blood plasma in a noncolligative fashion. That is, they lower the temperature at which ice will form but do not lower the melting temperature of the ice (DeVries 1983). The difference between the freezing and melting temperatures is termed thermal hysteresis. This noncolligative lowering of the freezing temperature is unique to the antifreeze proteins and forms the basis for their identification, purification, and quantification in plasma. The mechanism(s) whereby these antifreeze proteins prevent ice crystal growth is not well understood. However, it appears that the antifreeze proteins bind to the ice surface and somehow prevent the addition of water molecules to the ice lattice (DeVries 1983). DeVries (1980) has suggested that these antifreeze proteins may act at the epithelium and prevent the entry of ice crystals into fish.

In all of the species studied to date, the plasma antifreeze levels correlate with the seasonal period of lower water temperatures. Thus, the Antarctic *Trematomus* fishes and *Myoxocephalus scorpius* from the Artic retain high levels of plasma antifreeze throughout the year (DeVries 1980; Fletcher et al. 1982a), whereas fish such as the winter flounder (*Pseudopleuronectes americanus*), shorthorn sculpin (*Myoxocephalus scorpius*), and Atlantic cod (*Gadus morhua*) inhabiting the more temperate waters off Newfoundland, Nova Scotia, and Long Island, New York, contain little or no plasma antifreeze during the summer period of peak water temperatures (Fletcher 1977; Hew et al. 1980; Fletcher and Smith 1980; Petzel et al. 1980; Fletcher et al. 1982b).

We have recently characterized the antifreeze proteins in another member of the family Cottidae found in Newfoundland waters, namely, the sea raven (*Hemitripterus americanus*) (Slaughter et al. 1981). However, attempts to study the annual cycle of antifreeze protein levels in this species revealed remarkable individual variation. Some individuals produced significant quantities of plasma antifreeze during the winter months, while others produced little or none. This observation demanded a more detailed study, for it had not been observed in winter flounder, shorthorn sculpin, or Atlantic cod. However, sea raven are difficult to obtain from the northeast coast of Newfoundland, a region that approaches the northern geographical range of this species (Leim and Scott 1966). Therefore, we decided to study the annual cycle of plasma antifreeze activity in a more southerly population(Passamaquoddy Bay, New Brunswick) where sea raven can be found in abundance.

Materials and Methods

Sea raven were caught at approximately monthly intervals (October 1980 to December 1981) from Passamaquoddy Bay, New Brunswick, in water 50–100 m deep by a western otter trawl. Dragging time was approximately 20–30 min. When the fish were brought to the surface, they were immediately placed in tanks of continuously flowing seawater at surface seawater temperatures. Surface temperatures could be 3–4° above bottom temperatures during late summer and early fall. The fish were taken to the biological station at St. Andrews, New Brunswick, within 2–3 h and maintained in 2000- to 4000-L aquaria under ambient conditions of temperature and photoperiod. The temperature of the aquaria was maintained at the bottom seawater temperature where the fish were caught with a small refridgeration unit. In most cases, blood samples were obtained from fish 7 d following capture. A small number of the fish (5–10) sampled in February and April had been maintained in the aquaria for 1–2 mo. Approximately 75% of the fish sampled were females ranging from 300 to 5000 g. The males ranged from 400 to 2600 g. Bottom water temperatures were measured using a reversing thermometer.

Blood samples were obtained from a caudal blood vessel using plastic syringes equipped with 21- or 23-gauge needles and stored in heparinized Vacutainers (Becton Dickinson). Hematocrits were determined as soon after blood collection as possible and the red cells were separated from plasma using low-speed centrifugation (~4000 × *g*). *Plasma* Cl^- was determined using a chloride titrator (model CMT 10, Radio-

meter Copenhagen), plasma Na^+ by atomic absorption spectrophotometry (Fletcher 1975), and total proteins by the biuret method (Henry 1964). Plasma Na^+ values were corrected for the amount of Na^+ in the heparinized test tubes.

Estimations of the amount of antifreeze peptide activity present in the plasma were made using two methods, both of which are highly reproducible. In the first method the total plasma freezing point depression was measured in duplicate using a freezing point osmometer (model 3D or 3R, Advanced Instruments Inc., Massachusetts), and the antifreeze component of the total plasma freezing point depression was obtained by subtracting the electrolyte contribution (NaCl) (Fig. 2). This method has been well established as giving a reasonably good approximation of plasma antifreeze activity in winter flounder (Duman and DeVries 1974; Fletcher 1977; Fletcher and Smith 1980; Fletcher 1981). However, the results obtained using this method in the present study suggested that there was antifreeze activity in sea raven plasma during the summer months (AFP-FPD, Fig. 2). To confirm this observation the melting and freezing temperatures of the plasma were determined in triplicate using a Clifton nanolitre osmometer. In this method the ice crystals are observed directly. The freezing temperature is the temperature at which the ice crystals grow, and the melting temperature is the temperature at which the ice crystals shrink. Thermal hysteresis, a measure of antifreeze peptide activity, is calculated from the difference between the melting and freezing temperatures (DeVries 1982). Further evidence for the presence of antifreeze peptides can be obtained by observing the ice crystal growth. When antifreeze peptides are present, growth of the ice crystal is in the form of long spicules rather than the hexagonal growth that occurs in salt solutions (DeVries 1982).

Results

Plasma Na^+ and Cl^- concentrations were at their highest values during the months of January to April. Their concentrations declined from this peak to reach minimal values during August and September (Fig. 1).

The mean freezing point depression of the plasma was greatest during the winter months of February to May and lowest during August and September (Fig. 2). In contrast with the Na^+ and Cl^- concentrations, considerable variation in freezing point depression between individual fish was evident, particularly during October to May. During April, for example, plasma freezing point depression ranged from 0.57 to 1.3°.

Plasma freezing and melting temperatures also showed seasonal cycles that were essentially identical to those of plasma Na^+, Cl^-, and total freezing point depression (Fig. 2). The values for the melting temperature were consistently higher than the freezing point depression values obtained from the NaCl concentrations. Thus it would appear that the freezing point depression, attributable to NaCl, underestimates the colligative effects of the ions in the plasma by approximately 0.07°. The plasma freezing temperatures obtained using the Clifton osmometer were approximately 0.17° lower than those obtained using the Advanced osmometer (Fig. 2). In addition, the estimation of thermal hysteresis was approximately 0.05–0.25° greater than the antifreeze freezing point depression computed from the difference between total freezing point depression and the freezing point depression attributable to NaCl (Fig. 2).

A significant amount of thermal hysteresis was present in all samples throughout the year (Fig. 2). In addition, the ice

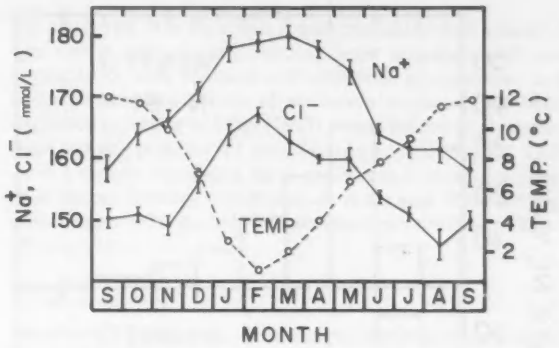

FIG. 1. Monthly changes in water temperature and plasma Na^+ and Cl^- concentrations in sea raven. Values are presented as means ± 1 SE. Number of animals at each point ranged from 12 to 48.

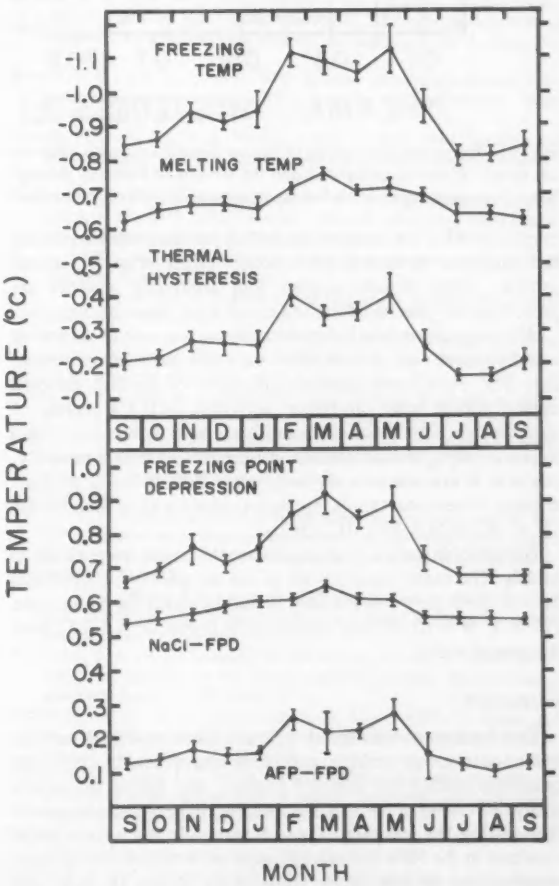

FIG. 2. Monthly changes in plasma freezing and melting temperatures, thermal hysteresis; freezing point depression, NaCl freezing point depression (NaCl-FPD), and antifreeze protein freezing point depression (AFP-FPD) in sea raven. All values are expressed as means ± 1 SE. Number of samples ranged from 12 to 48.

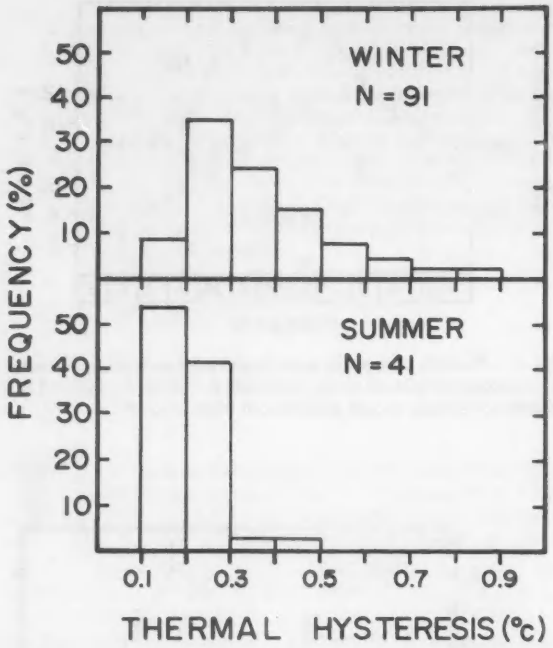

FIG. 3. Frequency distribution of plasma thermal hysteresis values in sea raven. Winter samples included the months of February through May. Summer samples included the months of July through October.

crystals in all of the samples, including the ones obtained during the summer, demonstrated spicular rather than hexagonal growth. These results indicate that antifreeze peptides are present in the plasma of the sea raven at all times of the year.

Although mean data indicated a winter increase in antifreeze activity, there was considerable variation between individual fish (Fig. 3). Clearly, almost half (45% of the fish sampled during winter have antifreeze activities (<0.3°) typical of summer fish. This variation in antifreeze activity was not related to sex or any gross anatomical differences in gonad maturity, nor was it correlated with body size. Total plasma proteins showed no seasonal trends; the mean value for all of the samples ($N = 183$) was 3.66 ± 0.1 (SE).

Hematocrits varied in an inconsistent fashion from month to month. The mean value for all of the samples ($N = 183$) was 20.1 ± 0.35. July was the only month in which the mean value (17 ± 1, $N = 6$) differed significantly (t-test, $P < 0.05$) from the annual mean.

Discussion

Two features of the annual cycle of plasma antifreeze activity in the sea raven are striking: antifreeze was present in significant quantities during the summer months, and during the winter many (40–50%) of the fish did not have higher antifreeze levels than during the summer. These features do not appear to be confined to the New Brunswick population of fish, for isolated observations of sea raven from Nova Scotia (6 fish) and Newfoundland (8 fish) have revealed identical information (Slaughter et al. 1981, and unpubl. data). Moreover, serial bleedings of two sea ravens (3–4 kg body wt) kept in laboratory aquaria at the same time under Newfoundland conditions of water temperature and photoperiod (Fletcher 1977) revealed that during the winter (January to April), one fish increased its plasma antifreeze activity levels by 0.5°, while the other only increased its levels by 0.1° (unpubl. data). It would appear that individual sea raven differ considerably in the amounts of antifreeze proteins they synthesize during the winter.

The results of the present study on sea raven contrast with those obtained for the three other species of temperate water fish so far studied: winter flounder, shorthorn sculpin, and Atlantic cod. All of these species contain little or no plasma antifreeze during the summer or fall period of peak water temperatures (Fletcher 1977; Hew et al. 1980; Fletcher and Smith 1980; Petzel et al. 1980; Fletcher et al. 1982b; Slaughter and Hew 1982). In Newfoundland, antifreeze proteins appear in the plasma of winter flounder and the shorthorn sculpin during November when the water temperatures approximate 4–6°C and reach peak values during January to March. The winter flounder begin to lose their antifreeze in May when the water temperatures usually rise above 0°C and they exhibit negligible values by late June. In contrast, shorthorn sculpin retain considerable quantities of plasma antifreeze until July and August long after any danger from freezing has passed. Antifreeze proteins do not appear in the plasma of Atlantic cod in any significant quantities until February, and they are lost from the plasma at approximately the same time of the year as they are in the winter flounder. In sharp contrast with the present observations on the sea raven, the plasma antifreeze levels of the winter flounder, shorthorn sculpin, and Atlantic cod showed very little variation between individuals of the same species (Fletcher 1977; Hew et al. 1980; Fletcher et al. 1982b).

To date, the maintenance of high levels of plasma antifreeze activity throughout the year has been a feature confined to fish species residing in polar and subpolar oceans. Antarctic fish (*Trematomus borchgrevinki* and *Rhigophila dearborni*) and shorthorn sculpin from the Arctic that inhabit waters that are near the freezing point all year round have high levels of plasma antifreeze during summer (DeVries and Lin 1977; Fletcher et al. 1982a). In addition, DeVries and Lin (1977) found that antifreeze levels did not decline in Antarctic fish when they were acclimated to 4°C for 60 d. DeVries (1980) has also observed that during the summer an Alaskan sculpin (*Myoxocephalus verrucosus*) only losses 50% of its winter levels of antifreeze despite the fact that the water temperature rises to approximately 7°C. Little is known concerning seasonal changes in antifreeze biosynthetic rates in polar fishes. However, the fact that the Antarctic and Alaskan species do not lose their antifreeze proteins, even when acclimated to relatively warm water, suggests that they are synthesized all year round (DeVries and Lin 1977; DeVries 1983). The results of the present study on New Brunswick sea raven suggest that this species also synthesizes antifreeze proteins throughout the year. Since the sea raven is a cottid, it is possible that its evolutionary origins, like those of the shorthorn sculpin, are in the Arctic where antifreeze peptides would be necessary throughout the year (Cowan 1972).

In winter flounder, antifreeze biosynthesis is a seasonal event (Hew et al. 1978; Hew and Fletcher 1979). It is synthesized by the liver prior to winter, and the control of antifreeze biosynthesis occurs at the level of mRNA transcription and possibly translation (Lin 1979; Davies and Hew 1980; Lin and Long 1980; Pickett et al. 1983). Although not studied in any detail, it is likely that antifreeze biosynthesis is also a seasonal event in Newfoundland shorthorn sculpin and Atlantic cod (Hew et al. 1980; Fletcher et al. 1982b). Studies on the winter flounder also

indicate that the timing and length of the antifreeze biosynthetic period are matched to the annual water temperature cycle of its geographical habitat and is to some degree genetically determined (Fletcher and Smith 1980; Petzel et al. 1980; Fletcher 1981). The observation that sea ravens maintain significant quantities of antifreeze proteins in their plasma during the relatively warm (12°C) summer months suggests that it does not have the genetic capacity to stop antifreeze biosynthesis.

Why do some sea ravens greatly elevate their plasma antifreeze levels above summer values while others residing in the same habitat do not? Very little is known about the biology of sea ravens. In Newfoundland they are rarely seen in the shallow (<15 m) inshore waters at any time of the year and we have no record of their presence in these waters during the winter. The bay in which the sea ravens of this study were caught is generally ice free, and at depths of 50–100 m there would be no danger of freezing. Most studies indicate that sea raven regularly inhabit relatively deep inshore waters (Merriman and Warfel 1948; Tyler 1971). There is one report of a sea raven egg mass being found at the high tide mark on Long Island, New York (Fuiman 1976). However, Warfel and Merriman (1944) indicated that the egg masses are commonly found in 18–30 m of water 1–3 mi (1.6–4.8 km) from the Rhode Island coast. Therefore, there appears to be no evidence that sea ravens would normally encounter ice-laden seawater at subzero temperatures. If sea ravens do not frequent icy seawater it is possible that antifreeze proteins are not essential to their survival. This would allow considerable genetic polymorphism with respect to antifreeze gene expression to develop within the population. Extensive research on the winter flounder has revealed that the antifreeze polypeptides are expressed by several genes (Davies et al. 1981). Therefore, as suggested by Pickett et al. (1983), it is conceivable that one gene is transcribed during the summer, whereas the others remain inactive. In the sea ravens that show large increases in plasma antifreeze levels during the winter, one or more additional genes may be transcribed. The mechanisms controlling antifreeze gene or genes expression in the sea raven should prove to be an interesting topic for future studies.

It is interesting to compare the two methods used in this study to estimate plasma antifreeze protein activity. For rapid routine measurements of freezing point depression, a standard freezing point osmometer, such as the Advanced instrument used in this study, is an asset. For example, one can measure samples at the rate of one per minute. On the other hand, direct ice crystal observation using the Clifton osmometer is at least an order of magnitude slower and is not without demands on patience and skill. We have found that as long as one pays little attention to "antifreeze" activity values of <0.1°, the standard freezing point osmometer, coupled with Cl^- estimations, gives a very reliable estimation of plasma antifreeze activity in winter flounder and shorthorn sculpin (Fletcher 1977, 1981; Fletcher and Smith 1980; Hew et al. 1980). On the other hand, this method is of no value with regard to the antifreeze glycoproteins found in cod (Hew et al. 1981), which can be readily detected using instruments such as the Clifton osmometer where the melting and freezing behavior of a small seed ice crystal is observed. Since antifreeze proteins also cause the ice crystals to grow in spicules rather than hexagonally, this method also has the advantage over a conventional freezing point osmometer in that it provides confirmation of the presence of antifreeze proteins (DeVries 1983). Although the standard freezing point osmometer did detect the presence of antifreeze polypeptides in our sea raven, it is clear that it underestimated their quantity. The reason why standard freezing point osmometers do not appear to be able to detect cod antifreeze glycoproteins and underestimate the activity of sea raven antifreeze has been explained in part by DeVries (1983). Some antifreeze glycoproteins and polypeptides are very sensitive to supercooling. That is, if a sample containing these antifreezes is supercooled by 4–6° before freezing is initiated, as is the case with freezing point osmometers, the freezing point depression will be reduced considerably.

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Variability in Hydroacoustic Biomass Estimates

Comment

In a recent paper, Kimura and Lemberg (1981) pointed out that the variance of hydroacoustic survey biomass estimates is strongly influenced by the patchiness of fish distributions caused by schooling. They use simulations to measure the contribution of schooling to the coefficients of variation of estimates associated with different shaped survey transects.

In this note I (a) discuss the reason that the transects vary in efficiency, (b) propose two alternative measures of sampling effort that allow a more realistic comparison between sampling methods, and (c) suggest that the measure of sampling intensity proposed by Kimura and Lemberg (SI_3) is inferior to one they discard (SI_1) as a means of comparing sampling in arbitrary regions.

These comments will be made in terms of hydroacoustic surveys but may be applied to other line transect density surveys and to line intercept surveys.

The effect of schooling on the variance of hydroacoustic biomass estimates may be described without postulating a specific model for fish distribution. This description clarifies Kimura and Lemberg's results and to some extent makes them predictable.

The patchiness of fish distributions is expressed mathematically by saying that fish densities (measured at approximately the same time) at two points close together are positively correlated. Usually this correlation will decrease with increasing distance and will be negligible beyond a certain minimum distance, which may be called the scale of patchiness. (In Kimura and Lemberg's simulations this scale is precisely the school diameter.)

In hydroacoustic surveys, the survey transect is divided into a number of equal intervals, and fish density for the region is estimated as the mean of the densities at all the intervals. The variance of this estimate will have a contribution from correlations between interval densities. The closer the intervals are to each other, the higher the contribution from correlations and thus the less efficient is a survey. Kimura and Lemberg considered three ways of constructing a transect from a number of traverses of a rectangular region: stratified (= equispaced) parallel, random parallel, and stratified (=equi-angled) zig-zag. Random parallel transects perform poorly because they allow the possibility of adjacent traverses being close and thus highly correlated. Stratified parallel transects minimize intertraverse correlations by maximizing the distance between traverses. With zig-zag transects there will be high intertraverse correlations at the vertices.

To decide which of two methods of constructing a survey transect is superior, transects that involve equal survey effort should be compared to see which has the lower variance. Kimura and Lemberg's comparisons are of transects with equal numbers of traverses which does not equate with equal effort when one transect is composed of zig-zag and the other of parallel traverses. The most plausible measure of survey effort is total distance covered. This gives rise to two alternative measures depending on whether the distance between traverses is included or not. In hydroacoustic surveys, effort is proportional to ship's time and should thus include intertraverse distances, but in some ecological surveys the effort involved in traveling between

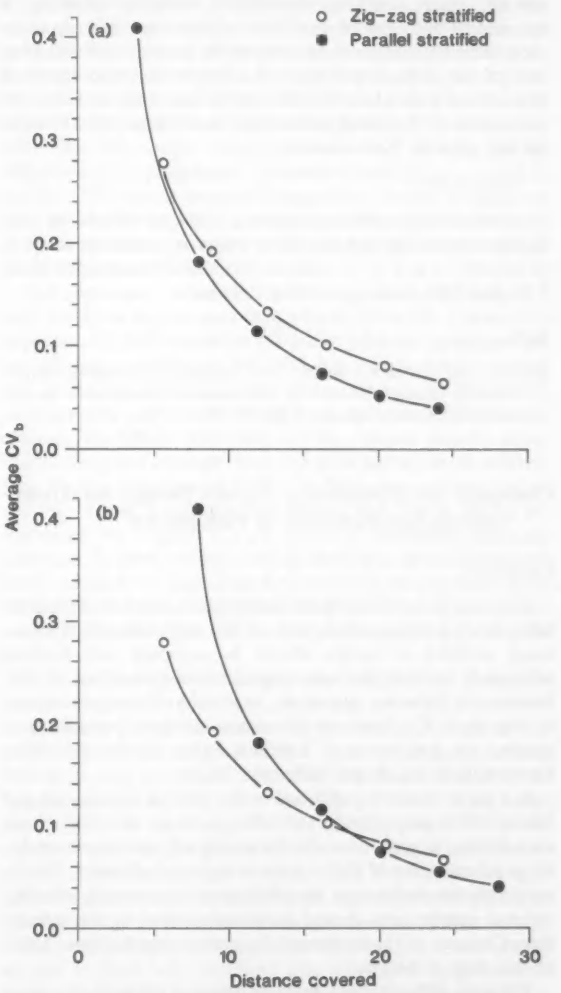

FIG. 1. Average CV_b (contribution of schooling to the coefficient of variation of the biomass estimate) as a function of distance covered (a) excluding and (b) including intertraverse distances. (Data from Kimura and Lemberg (1981), table 1a; schools covering 20% of a rectangular (1 × 4) region.)

traverses may be negligible compared with that expended in sampling.

The efficiencies of stratified zig-zag and parallel transects are compared in Fig. 1 using the two alternative effort measures given here. That the parallel method is uniformly superior when the intertraverse distances are not included should not be surprising in the light of the above comments on correlation.

Kimura and Lemberg recognized that to compare sampling in areas of different size a measure of sampling intensity is required. Their measure, which may be written as

$$SI_3 = 0.5T/A^{1/2}$$

(where A is transect area and T is transect length, measured with or without intertraverse distances), is inadequate for two reasons. First, if two surveys with the same A and T are joined, then the samping intensity of the resulting survey should be the same as that of the original surveys. With SI_3 it is greater. Second, since sampling intensity is, roughly speaking, a measure of the proportion of fish schools that is likely to be encountered by a transect, it is a property not only of T and A but also of the size distribution of schools. A scale invariant measure such as SI_3 would be appropriate only if, when the dimensions of the survey areas were doubled, so were those of the fish schools. The measure

$$SI_1 = T/A$$

is more suitable when comparing surveys where the size distributions of the fish schools are approximately the same in all surveys — R. I. C. C. Francis, *Fisheries Research Division, P.O. Box 297, Wellington, New Zealand.*

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Comment on Optimizing Survey Design for Determining Age Structure of Fish Stocks

Comment

The question of how many length measurements should be taken from a commercial catch of fish and subsequently how many otoliths or scales should be sampled and read to adequately estimate the age composition of the catch is of vital concern to fisheries scientists, especially those in resource management. Guidance on this matter has been provided by a number of authors (e.g. Ketchen 1950; Southward 1976; Kimura 1977; Smith and Sedransk 1982).

In a paper recently published in this journal Schweigert and Sibert (1983) purported to solve this problem. However, there are a number of problems with their analysis and, consequently, the practical value of their results is extremely limited. This is very unfortunate because the techniques they used for finding optimal sample sizes (based on formulae cited by the authors from Cochran 1977) are potentially quite useful for these kinds of sampling problems.

The main difficulty with these techniques, for finding optimal sample sizes (given in Cochran 1977 and in other sampling texts) is that one must have a fixed and known level of variance and a total cost before sampling takes place. The procedure is then to find an optimal sampling level that minimizes the expected cost for the fixed variance or minimizes the expected variance for the fixed total cost. In either case the researcher must have certain definite information about the variance structure of the population being sampled. This is usually a difficult thing to determine if little is known about this population. It may be possible to estimate the variances from a pilot study but the estimates of the optimal sample sizes will be subject to sampling error. Depending on the magnitude of the sampling errors these estimates can be highly unreliable.

Schweigert and Sibert (1983) have obviously missed this point. They have estimated the variance from the very sample for which the optimal sampling levels are being determined. The variance V in the product in their eq. 9, according to Cochran (1977), refers to the population variance and not the sample estimate as they have interpreted it. The authors' reference to eq. 10.34 in Cochran (1977) as the sample estimate is in error because this is the population variance, whereas the sample estimate is given in eq. 10.36 as

$$v(P_\ell \ldots) = (1 - f_1)s_1^2/n + f_1(1 - f_2)s_2^2/nm + f_1f_2(1 - f_3)s_3^2/nmk$$

where $f_1 = n/N$, $f_2 = m/M$, and $f_3 = k/K$.

This confusion is perpetuated by the authors' use of uppercase S for the sample estimate of the components whereas Cochran (1977) uses this notation to indicate population values. Therefore, Schweigert and Sibert (1983) have presented a crude "post facto" analysis. Their results may have same value in roughly evaluating what had been done but offer little information regarding future designs. Because one rarely knows what the age composition of a catch is before sampling and the age composition in the population can change over time, a sampling scheme that is optimal now may not be so in the future. In addition, if the authors intend on designing their survey such that it is optimal for one or a few age-classes, sample size alone will not necessarily guarantee high precision. Since the authors defined their variable for the presence or absence of a certain age-class according to a binomial model then the associated variance (S_3^2) can change by an order of magnitude depending on whether that age-class is highly abundant (or rare) or present 50% of the time.

Next, it is important to point out that the methods used by Schweigert and Sibert assume subsampling with units of equal size. That is, it has been assumed that each sample has the same number of fish, each load contains the same number of samples, and each vessel has the same number of loads. This assumption simplifies the calculations but rarely holds in practice. Techniques for dealing with unequal unit sizes are available in most sampling texts. They are more difficult to use and require estimators to be weighted to take into account the variable sizes of the units at all stages of the sampling procedure. However, the authors have not provided enough information for the reader to assess how well their equal unit size assumption approximates to the unequal unit size approach.

Finally, the authors make the assumption that the sampling fractions are negligible (Schweigert and Sibert 1983, eq. 4b). This assumption is used in the derivation of the optimum sample sizes but is contradicted in two places in the paper. In the paragraph immediately below eq. 3 the authors state that the number of fish taken in a sample (K) is on average 36% larger than those sampled for age (k). This implies that the sampling fraction k/K is on average 0.74, a value that is not negligible. Furthermore, for the examples presented in the Methods section

(p. 592) K is given as being equal to 100 fish and from Table 2, k′ (the calculated optimum sample size) ranges from 5 to 101. Thus, the assumption that sampling fractions are small is not at all appropriate in this case.

Equations 9–13 are presented by the authors to show the derivation of the solution of the optimal sample sizes. Their derivation is, however, incorrect and, in any case, is unnecessary. (If one was to substitute the solution for m' given in eq. 14 for m in eq. 13 as it is written the authors would have us believe that $2\sqrt{2} = 1$.) It is unnecessary to present the derivation because the solution to this problem has been given in more general terms by Cochran (1977, p. 288, eq. 10–44) as

$$(1) \quad m' = \frac{\sqrt{S_2^2 - S_3^2/K}}{\sqrt{S_1^2 - S_2^2/M}} \times \sqrt{C_1/C_2}$$

$$(2) \quad k' = \frac{S_3}{\sqrt{S_2^2 - S_3^2/K}} \times \sqrt{C_2/C_3}.$$

Equations 14 and 15 in Schweigert and Sibert (1983) are a limiting case of (1) and (2) presented here by letting K and M tend to infinity.

As noted above, values of K encountered by the authors are not large and therefore optimal values for m and k should be calculated from (1), perhaps with modifications for large M, and (2), respectively. The implications of these changes would be that m' will be smaller than calculated in the paper and k' will be larger. For example, the values for k' and m' in Table 2 for age 2 (Barkley Sound) for the first cost structure would be 118 (117.6) and 0.4, respectively, as compared with the values of 42 and 1 calculated by the authors.

I hope that the authors will reconsider their approach to the optimal sample size problem in light of the comments raised here — S. J. Smith, *Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2.*

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Reply

S. J. Smith (1984) offers several criticisms of our recent paper on optimizing sampling design. However, it is evident from the first statement of this comment that he has misinterpreted the purpose of our paper. He does present a number of interesting comments that we will attempt to address in detail below.

Our objective was not, as the comment suggests, to estimate age composition by a stratified sampling design based on the length composition of the catch (Ketchen 1950; Smith and Sedransk 1982; etc.). We are well aware of the myriad of papers that deal with this subject and had no desire to replicate this work. Our objective was to present a simple methodology that could be used, given information on the age composition from several samples from the catch, to estimate the most cost effective means by which to sample the total catch by the commercial fishery. Fisheries operate in a number of areas on a variety of stocks whereas stock assessment and management decisions are usually based on genetically discrete units requiring accurate estimates of their age structure and other biological attributes, on the appropriate geographical scale. Hence, it is imperative to obtain the most accurate and precise estimate of the age composition for each of these units possible with the minimum sampling effort. If stratified subsampling by length were a desirable attribute of the sampling design it could readily be incorporated and its effects on overall efficiency and precision evaluated (Cochran 1977, p. 288).

Smith's main criticism of our paper stems from the way in which we have expressed total variance. Our equations 4a, 4b, and 9 all refer to population variances. In fact, the formulae we have presented should all be population parameter estimators. As such we may have confused some of our readers, since the n, m, and k in equations 5, 5′, 6, 7, and 7′ should be capitalized to reflect the fact that the S_i^2 are population variance estimators. Note that the sample estimators are identical save for the additional finite population correction factors (f_1, f_2, and f_3 in Smith's (1984) first unnumbered equation). Hence, although we have presented the equations for population parameters, our analyses were done using the appropriate sample estimators (eq. 10.36 of Cochran 1977).

The importance of whether to use variance estimates from a pilot survey or not has been exaggerated by Smith. Usually it is impractical in field situations to conduct pilot surveys to provide several variance estimates with which to reduce sampling errors and derive the optima for the main surveys. In this event, Cochran (1977, p. 284) provided formulae with which one may estimate confidence intervals for the optimal sample sizes, should the point estimate from the pilot survey be unsatisfactory. Further, as Cochran (1977, p. 283) demonstrated and as our Fig. 1 and 3 indicate (Schweigert and Sibert 1983), the procedure we suggest is very robust to deviations from the optimum. In other words, although there may be sampling error in the variance estimates from a pilot survey, the estimates of the variance from a survey using these derived values of the optimal m and k should still be similar to that from a survey using the true optima.

Next, as Smith points out there obviously will be changes in the age composition over time, which may result in a suboptimal design for some age-classes during some years. Clearly, as we emphasized in our original work, there are constraints, both biological and logistical, that make the determination of an optimal design for all age-classes in all areas in all years impossible. We mentioned the fact that an optimal design for one age-class would not be so for others because of the different relative abundance of age-classes. Also, there is the fact that different areas may have different age compositions so an optimal design in one area would not be so in another.

One would hope, however, that this analysis would not be an end in itself but something that would be reevaluated on an iterative basis as new information became available. A researcher should have some intuitive feeling or historical information about the range of variability in the age composition of the stocks under investigation that could be incorporated into the design directly, or through a simple simulation model.

Indeed, large areas of statistics are devoted to the analysis of the problem of using prior information to improve current estimates.

The rationale for assuming equal rather than unequal sized sampling units was twofold. First, there was no good information available from which to calculate the true sampling unit sizes. Hence, this is the simplest assumption and it does not unnecessarily complicate the derivations and analyses. Our analysis is based on the average number of samples per load in the average landing in each area. It follows from this that the assumption of negligible sampling fractions is justified for N and M where they are of the order of 0.001 and 0.05, respectively. Smith correctly notes that the sampling fraction for k, 0.74, is not negligible.

This point is somewhat unclear, however, because K cannot be explicitly defined. In our usage, K is the number of fish in a sample and k is the number of fish actually aged. In practice, the difference between K and k occurs because it is often impossible to determine the age of all K fish. Therefore, K is usually chosen sufficiently large to ensure that at least k fish are obtained that can be aged. For an abundant fish such as herring, sampled by a two-stage sampling design, K could clearly be made as large as an entire load. In principle, therefore, K could be made much larger than k.

We were well aware of the optima as presented by Cochran (1977, p. 288, eq. 10.44) and in fact used them in our early analyses. Unfortunately, if S_2^2/M or S_3^2/K are large relative to S_1^2 or S_2^2, his equations will not yield estimates of m or k. Hence, we found that the derivations we presented assuming negligible sampling fractions were more useful for someone starting a survey who has no concept of what the optima may be and who must rely on the results of a pilot survey as we have done. In this case, Cochran's eq. 10.44 may provide the investigator with no information, since undefined m or k (square root of negative number) are not very useful for designing a survey. Hence, although our assumption of negligible k/K may not be warranted here, clearly our equations 14 and 15 at least provide crude optimal estimates that can be used to guide future surveys and deter excessive sampling in the process.

Unfortunately, we permitted a typographical error in our equation 13 to go uncorrected. It should read

$$m^2 - 2m\sqrt{\frac{C_1 S_2^2}{C_2 S_1^2}} + \frac{C_1 S_2^2}{C_2 S_1^2} = 0.$$

This would permit Smith to substitute m' in equation 14 for m in 13 and find that $\sqrt{1} = 1$. This "unnecessary derivation" was included at the insistence of a referee.

Finally, Smith presents some results of calculating optimum sample designs using formulae from Cochran (1977). His result for k' (from equation 1) is larger than ours by a factor of 3. This difference is certainly large but given the rather flat regions around the optima, particularly in the direction of larger samples (Schweigert and Sibert 1983, Fig. 2 and 3), either estimate of k' would produce a reasonably efficient sample design. Smith's result for m' (using an unspecified value of M in equation 1) is 0.4, which of course must be rounded to 1 making it identical to ours. Cochran (1977) also noted that optima calculated by these methods tend to be rather wide so that extreme accuracy is not called for. Smith's calculations, while numerically different from ours, support one of our main conclusions, that three-stage sampling is not necessary. A single sample from a load ($m = 1$) is sufficient. The real utility of this method will be determined by future applications to similar sampling problems — J. F. Schweigert, *Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6*, and J. R. Sibert, *South Pacific Commission, P.O. Box D5, Noumea Cedex, New Caledonia.*

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Singular, Mass-Specific P/B Ratios Cannot be Used to Estimate Copepod Production

Comment

Tremblay and Roff (1983) have made an admirable effort to estimate copepod production over a large area with important fisheries. Their values may indeed be closer to reality than are previous ones cited by them. However, this is uncertain, as they have used incorrect assumptions and analyses, which we discuss briefly here.

Potential P/B ratios for copepods are not mass-specific

The key assumption in the estimates by Tremblay and Roff (1983) is that annual production/biomass ratios for copepods are negatively related to adult body mass (M in kilocalories) by

$$\log P/B = -0.16 - 0.34(\log M).$$

This equation, which has the plausibility of many similar "surface laws" in biology, is from Banse and Mosher (1980) and is based on data from 32 invertebrate species ranging from approximately 15 μg to approximately 3 g. In their extensive analyses Banse and Mosher (1980) are much concerned with residuals in such relationships, and they caution against their use for specific groups and circumstances. We begin by asserting that the above equation is not merely imprecise in parameters but entirely wrong in form as a basis for estimating copepod production.

A P/B ratio has the dimensions of 1/time. Among copepods, the time taken for relative growth increments (e.g. over a stage, generation, etc.) is evidently unrelated to body mass when development is continuous and food abundant. This has been shown in predicted and observed generation lengths of five copepods in Loch Striven, Scotland (McLaren 1978). There, the tiny *Oithona similis* has generation lengths similar to those of the largest species sampled, *Calanus finmarchicus*. A similar conclusion can be gleaned from many published data on laboratory rearings. A good series is in Landry (1983), who reported times from hatching to maturity (15°C, food satiation) and body weights for *Rhincalanus nasutus* (25.4 d, 100–200 μg), *Calanus pacificus* (19.8 d, 70 μg), *Pseudocalanus* sp. (21.1 d, 10 μg), *Acartia clausi* (21 d, 2 μg), *A. tonsa* (20.3 d, 4 μg), and *Paracalanus parvus* (18.6 d, 3 μg).

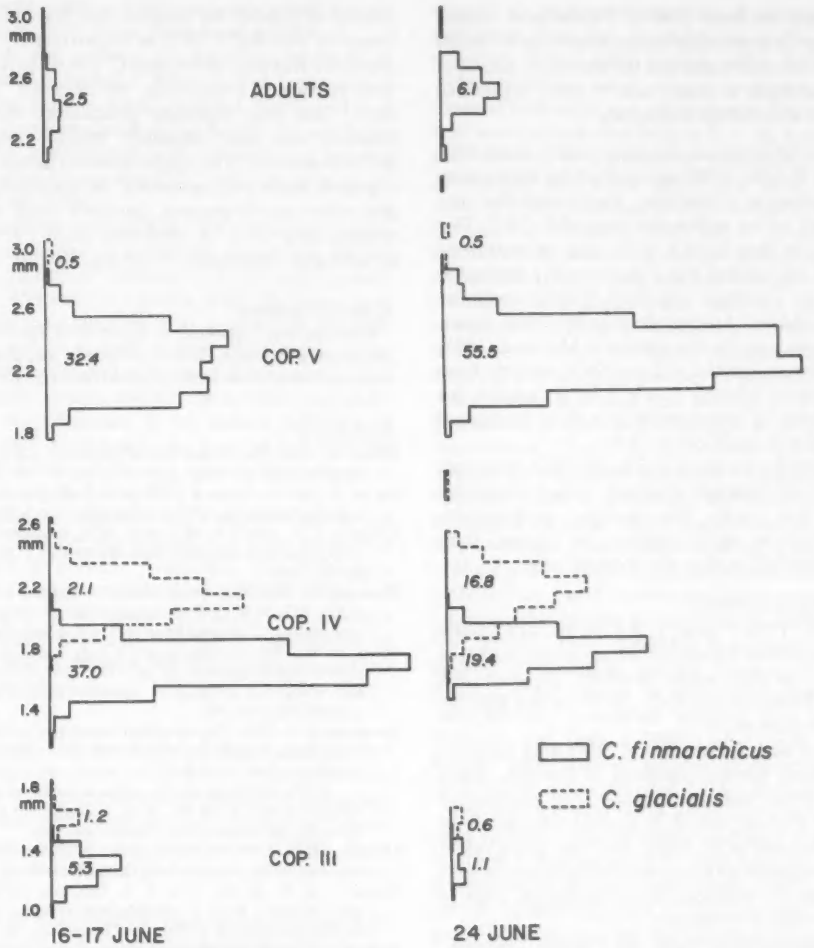

FIG. 1. Cephalothorax length (to the nearest 0.1 mm) of copepodid instars of *C. finmarchicus* and *C. glacialis* in combined samples from a repeated transect (eight stations) extending south of Cape Sable Island, N.S. Species were identifiable from fifth legs (Grainger 1961) in cop. V but were discriminated by fitting normal curves (Macdonald and Pitcher 1979) to the bimodal size distributions in cop. IV and V. Numbers are percentages of each stage of both species in the samples.

Given the independence of development rates on body size, it is possible that larger relative growth increments between stages among smaller species could produce a size-dependent *P/B* ratio. Any casual survey of the copepod literature will reveal no such relationship; if anything, the opposite is true. For example, the ratio of cephalothorax length to egg diameter in the large *C. finmarchicus* is approximately 17:1 (Corkett and McLaren 1970, table 3) compared with 9:1 in *O. similis* (data in McLaren 1969). Growth in later stages, which certainly supply most production, is probably more germane: *C. finmarchicus* increases in length approximately 2.1× between copepodid III and adult female (Grainger 1963, fig. 8) compared with 1.5× in *O. similis* (McLaren 1969, table 6). On this basis, it seems highly improbable that the *P/B* ratio of *O. similis* would be 4.8× that of *C. finmarchicus*, as implied by Tremblay and Roff (1983, table 1).

Of course the relationships explored by Banse and Mosher (1980) are for annual, not instantaneous, *P/B* ratios, although they conclude that the ratios differ little in relation to "continuous" vs. "non-continuous" life cycles. It is perhaps true that small copepod species in temperate marine waters tend to have continuous life cycles, at least during a part of the year, whereas large ones may be monocyclic (annual). This pattern might produce a negative relationship between annual *P/B* ratios and adult size, but it is unlikely to be predictable from the very general regression used by Tremblay and Roff (1983).

This lack of dependence of development rate, specific growth rate, and by implication *P/B* ratios on body size in copepods has

already been stressed by Frost (1980). Furthermore, Banse (1982), following up on some reservations expressed by Banse and Mosher (1980), has concluded that the mass dependence of growth rate may not apply to major taxa of small organisms, including Cladocera and perhaps Copepoda.

Potential P/B ratios for copepods are temperature dependent

While Banse and Mosher (1980) recognized the temperature dependence of short-term production, they concluded that annual *P/B* ratios are not so, within the range of 5–20°C. This conclusion is based on data from a wide range of organisms from many regions and habitats but is questionably applicable on the scale used by Tremblay and Roff (1983). Certainly, potential development rates of copepods are temperature dependent. Solutions of equations for five species in McLaren (1978) give ratios of generation lengths at 5 and 20°C ranging from 1:3.4 in *Pseudocalanus minutus* to 1:6.5 in *O. similis*. An example of estimation of temperature-dependent production rate is given by McLaren and Corkett (1981).

Since, as recognized by Tremblay and Roff (1983), copepods on the Scotian Shelf are markedly seasonal, failure to consider temperature could bias results. For example, in seasonally successional but otherwise similar species, the "spring" form might have fewer generations than an "autumn" one.

Nonproductive copepod biomass

Banse and Mosher (1980, table 1, fig. 4) modified some annual *P/B* ratios to account for species that spend much time as eggs or, conversely, as older stages or adults. However, the regression used by Tremblay and Roff (1983) did not take the latter correction into account.

The importance of such corrections in estimating instantaneous production rates among copepods is obvious. Adult copepods cease to grow, although females may continue to produce egg matter at a rate similar to specific growth rate of younger stages (Sekiguchi et al. 1980; McLaren and Corkett 1981). Adult males probably produce only insignificant amounts of matter and might be excluded from biomasses used to estimate production from *P/B* ratios.

In addition, copepods may enter an "active diapause" (Elgmork 1980), sometimes for long periods, when the instantaneous *P/B* ratio is accordingly zero. As noted by Banse and Mosher (1980), such life history features will influence estimates of mean biomass and *P/B* ratios on an annual basis. An example of the problem is evident in samples of the sibling species *C. finmarchicus* and *C. glacialis* (Fig.1) from the fringes of the area sampled by Tremblay and Roff (1983). During the week between the two sample periods, mean stage in *C. finmarchicus* increased by about one-half stage, whereas *C. glacialis* was clearly "stalled" in copepodid IV. The latter species was probably diapaused and is evidently univoltine even in these waters at the southern limit of its range.

Inadequacies of mass as a measure of copepod size

In applying *P/B* ratios to estimate production, it has to be assumed that mass (or energy) units are equivalent among species. A pitfall in such an assumption is hinted at in Fig. 1. In copepodid IV, where both species are well represented, *C. finmarchicus* averages 1.71 mm and *C. glacialis* 2.14 mm in cephalothorax length (fitted means, both samples combined). This length difference of approximately 25% is similar to the difference in adults in various arctic and subarctic localities (modal difference of 30% from Grainger 1963, table 1) and suggests that bodies of the larger species are about twice the volume of those of the smaller. Yet, body masses are listed by Tremblay and Roff (1983) as approximately 5× greater in *C. glacialis*. This may be because *C. glacialis*, like other copepods with diapausing copepodids, stores much "oil" (wax esters, etc.). This does represent production, but not productive biomass, and may ultimately become largely used up in self-maintenance. The epiphenomenal nature of stored "oil" in copepods needs to be accounted for in applying *P/B* ratios, and also other size-dependent functions such as metabolic and feeding rates — I. A. McLaren and C. J. Corkett, *Biology Department, Dalhousie University, Halifax, N.S. B3H 4J1.*

Acknowledgments

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Reply with Additional Notes on *P/B* Ratios

McLaren and Corkett (1984) argue that the BM equation (Banse and Mosher 1980) used by us (Tremblay and Roff 1983a) is wrong in form. They are essentially correct. However, many ecological relationships or functions are wrong in form or dimensions but are nonetheless empirically useful, for example the morphoedaphic index (Ryder 1965). That strong relationships exist overall between body size and life history parameters is beyond question (Blueweiss et al. 1978; Banse and Mosher 1980; Humphreys 1981; Peters 1983). That such relationships

may not strictly apply within a given taxonomic group appears to be the case (Banse 1982; Frost 1980; Landry 1983).

Our position was, and remains, that the BM equation gives reasonable estimates for annual P/B ratios, and hence annual production, for the Scotian Shelf copepod species. It will become apparent, however, that this may well be because body size is ecologically confounded with temperature, arrested development, "nonproductive biomass," etc., as detailed by McLaren and Corkett (1984).

In our paper (Tremblay and Roff 1983a) we were interested in deriving realistic estimates of secondary production for the Scotian Shelf. A variety of methods exist for making such estimates (see e.g. Winberg 1971; Greze 1978). However, most methods require more information than is generally available from temporally widely spaced cruises over broad areas. Typically, zooplankton data are only reported as biomass, at its crudest, displacement volume, and at its best, instar analysis by species, but still only biomass. If we require estimates of production when data are limited, we may be left with the application of a P/B ratio as the only option. The question is how should these be derived or applied?

The basic argument of McLaren and Corkett (1984) is that P/B ratios are not mass-specific and that P/B ratios so derived should not be used to estimate production. The differences between their position and ours (Tremblay and Roff 1983a, and this paper) concern two major issues: (1) the degree of accuracy expected of production estimates and (2) the application of laboratory data to field conditions and the confounding effects of environmental variables.

Degree of accuracy

Tremblay and Roff (1983a) followed Banse and Mosher (1980) in suggesting a precision of 50–200% of the production estimate. There is no doubt that other methods of estimating secondary production yield more precise (although not necessarily more accurate) results, e.g. Rigler et al. (1974). Such abundant data as presented in Rigler et al. (1974) are rarely available.

The application of Belehradek's function (as advocated by McLaren 1978) to calculate generation times requires knowledge of the environmental thermal history of a species, combined with laboratory-measured developmental rates. For several species there may be no consistent pattern of vertical distribution or relationship to temperature (Turner and Dagg 1983), and thus, calculation of in situ development rate is uncertain. Where environmental and species data are sparse the application of growth functions also leads to inaccuracies or wide limits of precision.

Estimates of production based on P/B ratios

We must consider two questions: (i) are production estimates based on (mass specific) P/B ratios appropriate? and (ii) are our production estimates for the Scotian Shelf (Tremblay and Roff 1983a) realistic?

First, we reiterate that there is good correspondence between our mass-specific P/B estimates and those derived from the literature (see Tremblay and Roff 1983a). Indeed, we could simply have applied P/B ratios selected from the literature to our data, but this would have left us without production estimates for several species. To adduce independant evidence we now follow other lines of empirical argument.

Relationships between body size, generation time, and number of generations per year

Under similar laboratory regimes of temperature and food there is no relationship between development rate and hence generation time (egg to adult) and body size among pelagic copepods (Frost 1980; Landry 1983; McLaren and Corkett 1984). However, under natural conditions, other factors confounded with body size affect development rate. Daily growth rates referenced to temperature (e.g. by Belehradek's function) only allow us to calculate the maximum expected number of generations per year. Departures from isochronal growth (Landry 1983), diapause in late copepodites (notably *Calanus hyperboreus* (Conover 1962) and *C. glacialis* (McLaren and Corkett 1984)), and refractory periods between maturation and reproduction (in *Pseudocalanus minutus* and other species (McLaren 1978; Carter 1969; Roff and Carter 1972)) are not accounted for. Despite McLaren's (1978) observations, other studies (e.g. Evans 1977) show significant differences between observed and predicted number of generations.

We should note also that separation of "populations" into generations is often a difficult task even given frequent field observations. Thus, Marshall (1949) and Davis (1982) both claimed that reproduction in *Oithona similis* is continuous, with indistinct peaks of production and ovigerous females present throughout the year. Depth distributions may also be bimodal, with animals in deeper water developing more slowly.

These problems are compounded over broad open areas such as the Scotian Shelf because planktonic populations at different stages of development are advected by currents (Tremblay and Roff 1983b). Thus, sampling at a specific location may not disclose interpretable cohorts because of the influence of different water masses. In restricted bodies of water, such as Loch Striven, generation analysis may be much simpler. McLaren and Corkett (1984) also argue "no relationship" between body size and number of generations per year from Loch Striven data (McLaren 1978; Marshall 1949).

The final test here is the ecological data set itself. We took adult weights from Tremblay and Roff (1983a, and unpubl. data) and J. Anning and J. C. Roff (unpubl. data) and numbers of generations per year from Evans (1977), McLaren (1978), and M. J. Tremblay and J. C. Roff (unpubl. data). Using these data (Table 1) we ran several regression analyses on the relationship between body size and number of generations per year. We specifically included both "worst" and "best" cases (i.e. highest number of generations for larger species and lowest number for smaller species and vice versa). Note that some of these species are not important on the Scotian Shelf but that we have *not* included several small neritic or estuarine temperate species with high numbers of generations per year (whose inclusion favors the relationship).

Our "worst case" analysis ($\log_{10}$ transformed data) accounted for 74.3% of the variance ($P < 0.001$) and our "best case" for 78.8% ($P < 0.001$). Thus, whatever factors may influence the number of generations actually achieved per year under field conditions, the significance of body size as a strong correlate of number of generations per year is demonstrated. Undoubtedly this is mainly because size and temperature distributions in copepods are ecologically confounded, with a strong tendency for larger species to inhabit deeper colder waters and therefore to have longer generation times.

Relationship between daily P/B and body size

One method widely used to estimate seasonal production rates involves first estimating daily P/B ratios. Greze (1978) stated "...most planktonic animals can produce from 0.05 to 0.15 times their biomass per day...". Assuming a production season of approximately 180 d (April–September), this trans-

TABLE 1. Data used for regressions of body size and number of generations per year in copepods. (Data are from Evans (1977), McLaren (1978), Tremblay and Roff (1983a, and unpubl. data), and C. J. Corkett, pers. comm.).

Species	Adult weight (kJ)	No. of generations per year
Calanus hyperboreus	0.069	1
Calanus glacialis	0.031	1
Calanus finmarchicus	0.0056	1.5–3
Metridia lucens	0.0022	2–3
Centropages hamatus	0.00069	6–8
Pseudocalanus minutus	0.00023	4–6
Oithona similis	0.000047	4–6
Microcalanus pygmaeus	0.00011	4
Temora longicornis	0.00034	5–6
Acartia hudsonica (= *clausi*)	0.00019	6–8

lates to an annual P/B range of 9–27. This is very similar to the annual P/B range that we calculated (6.6–31.7) (Tremblay and Roff 1983a) from the BM equation, with the exception of the single generation species *C. hyperboreus* and *C. glacialis*.

We also regressed the data summarized by Greze (1978, table 3) of body weight and daily P/B. There is a highly significant relationship ($R^2 = 87.9\%$, $P < 0.001$). Thus, whatever variables determine the relationship (again presumably mainly the effect of temperature), they are strongly correlated with body size.

P/B ratios within generations

A substantial body of information on a wide variety of both pelagic and benthic species suggests that P/B ratios per generation normally lie between 3 and 6 (e.g. Waters 1969). Even under extreme conditions of low temperature and oligotrophy the P/B ratio for the copepod *Limnocalanus macrurus* in Char Lake, N.W.T., was still 2.5 (Rigler et al. 1974; J. C. Roff, unpubl. biomass data). A normal P/B of about 4 (range 3–5?) is probably acceptable for many zooplankton species.

The relationship between body size and P/B per generation is not yet clear. Although predation on zooplankton is often size selective (e.g. Brooks and Dodson 1965), this is not the case for "generalist" feeders (L. Coates-Markle and J. C. Roff, unpubl. data), and overall size effects on zooplankton communities may not be strong (Roff et al. 1981). Thus, within zooplankton communities the relationship between size and mortality rates is not established (but see Myers and Runge 1983). However, the hypothesis that mortality rate (including predation) and hence its influence on P/B per generation is inversely mass-specific in a given taxonomic zooplankton group under natural conditions is not unreasonable. Also, Waters (1969) and Mathews (1970) showed that cohort P/B is closely related to instantaneous growth rate. Thus, larger organisms, at lower temperatures, experiencing a lower rate of predation would be expected to have fewer generations per year (as demonstrated) and also a lower P/B per generation. A combination of slow development rate and low predation (Roff and Carter 1972; Rigler et al. 1974) would explain the low P/B ratio for *L. macrurus* in Char Lake.

Scotian Shelf production estimate recalculated

We next recalculated our production estimates for the Scotian Shelf (Table 2), including only those species for which we have independant data on number of generations per year under appropriate conditions. Production was calculated (1) using a size-invariant cohort P/B of 4 and (2) using a size-variable P/B value assigned as a whole number based on $\log_{10}$ of body weight (we have no evidence to support this but see Waters 1977 and our hypothesis above).

The data show good consistency (Table 2), and without exception the recalculated production values fall within the range of our original estimates (Tremblay and Roff 1983a) for copepod species on the Scotian Shelf. Even in our "worst case" analysis (constant cohort P/B, number of generations furthest from agreement with the BM equation), our recalculated values still lie within the 50–200% limits of our original estimate. There is a probability that *C. finmarchicus* may be more productive and *O. similis* less productive than we originally estimated (Tremblay and Roff 1983a).

It also appears likely that production of several smaller neritic species may be adequately predicted from the BM equation. Thus, annual P/B ratios for *Acartia hudsonica* (= *clausi*) in Passamaquoddy Bay (K. Middlebrook and J. C. Roff, unpubl. data) are 20 from the BM equation and 18–28 from Belehradek's function and agree well with other P/B ratios in the literature for this species including Uye (1982).

Conclusions

Our defence has necessarily been an empirical one because McLaren and Corkett (1984) are essentially correct that the BM equation (Banse and Mosher 1980) is "wrong in form." This is because it does not explicitly include functions for temperature–growth interactions, delayed development effects, "nonproductive" biomass, etc. Probably because of the environmentally confounded nature of these parameters, especially body size and temperature distributions, the BM equation does empirically yield reasonable estimates for P/B ratios and hence production. In fact, what McLaren and Corkett (1984) have done is not to discount the utility of the BM equation, but rather to present several reasons why it may yield reasonable estimates. However, since the BM equation is at variance with laboratory-derived data, and in light of Banse's (1982) conclusions on mass dependance of maximal growth rates in smaller organisms, predictions using such equations should be cautiously interpreted.

The present debate between McLaren and Corkett (1984) and ourselves is reminiscent of the exchange between Hynes and Coleman (1968) and Hamilton (1969), which stimulated a refinement and simplification of secondary production estimates for stream benthos. In view of the considerable interest in both particle size spectra and production in pelagic and benthic ecosystems, we believe that more attention should be given to *ecological* relationships between size and production. — J. C. Roff, *Zoology Department, University of Guelph, Guelph, Ont. N1G 2W1*, and M. J. Tremblay, *Department of Fisheries and Oceans, Halifax, N.S.*

Acknowledgments

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TABLE 2. Recalculation of copepod production for the Scotian Shelf. Not all species from Tremblay and Roff (1983a) are included here. (Data from Tremblay and Roff (1983a, and unpubl. data) and Table 1; also see text for explanation.)

	Tremblay and Roff (1983a)				Constant P/B			Size-variable P/B		
	Mean annual biomass ($kJ/100\,m^3$)	P/B	Annual production ($kJ/100\,m^3$)	No. of generations per year	Per generation	Annual	Annual production ($kJ/100\,m^3$)	Per generation	Annual	Annual production ($kJ/100\,m^3$)
Calanus hyperboreus	4.85	3.0	14.5	1	4	4	19.4	3	3	14.5
Calanus glacialis	1.67	3.9	6.5	1	4	4	6.7	3	3	5.0
Calanus finmarchicus	39.75	6.6	262.4	1.5–3	4	6–12	238.5–477.0	3	4.5–9	178.9–357.8
Metridia lucens	8.39	9.2	77.2	2–3	4	8–12	67.1–100.7	3	6–9	50.3–75.5
Pseudocalanus minutus	2.69	19.1	51.4	4–6	4	16–24	43.0–64.4	4	16–24	43.0–64.6
Oithona similis	2.47	31.7	78.3	4–6	4	16–24	39.5–59.3	5	20–30	49.4–74.1
			490.3				414.2–727.1			341.1–591.5

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Reply

My comments on McLaren and Corkett's (1984) criticism of the paper by Tremblay and Roff (1983) addressed principally the potential readers of papers such as those by Banse and Mosher (1980), Tremblay and Roff (1983), or Western (1983): Empirically derived, interspecific equations tend to permit great deviations from the predicted values for individual species. This point, often forgotten by users of such "laws," is illustrated by the semilog plot in Fig. 1 of eq. 3 by Banse and Mosher (1980; cf. the more innocuous double-log presentation in their fig. 2). This equation and the underlying approach are at issue in the comment by McLaren and Corkett (1984).

First I wish to repeat the *caveat* in Banse and Mosher (1980, p. 371) that our equations not be used for species dominating the biomass of an assemblage. In the data of Tremblay and Roff (1983), however, *Calanus finmarchicus* contributed 60% of the annual mean biomass of the copepods. Any marked deviation of *C. finmarchicus* from the rule (cf. for the morphologically and behaviorally similar *C. pacificus* (Frost 1980)) would not only affect the predicted production of the entire assemblage but also the percentage contributions by the constituents. I note too that in our fig. 2, confidence intervals for our estimating eq. 3 were not given, a point overlooked by Tremblay and Roff. Thus I am in some agreement with the criticism of this paper by McLaren and Corkett (1984).

However, I am not as convinced as McLaren and Corkett (1984) seem to be that P/B ratios are not mass-specific. In my view, developmental or specific growth rates *only imply* similar P/B ratios. While the former have the dimension 1/time, the P/B ratio is the dimensionless quotient of cumulative (finite) production over mean biomass. The quotient only implicitly comprises time, a point not emphasized in Banse and Mosher

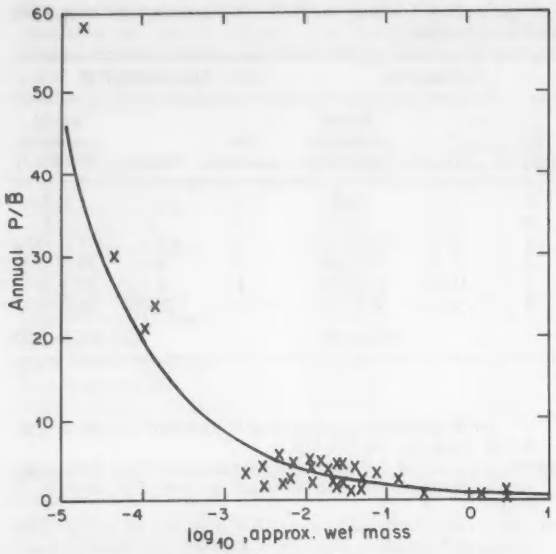

FIG. 1. Annual P/B as a function of mass at maturity for cool- and warm-temperate field populations of invertebrates. (Redrawn from Banse and Mosher 1980, fig. 2.)

(1980) and overlooked by Corkett and McLaren (1984); therefore, the link between those rates and the annual P/B might not be straightforward. For example, similar specific growth rates would yield similar P/B values only if the mortality patterns were similar (cf. the argument by Waters 1969, p. 173). Once potential developmental rates (or maximal intrinsic growth rates) are involved, as with McLaren and Corkett (1984), the connection may be even more remote; certainly, the interplay between generation length and annual P/B value is contrary to expectations (cf. figs. 3, 4 in Banse and Mosher 1980).

Hence, (1) it remains to be seen whether or not a measure of adult body mass is correlated with P/B quotients in pelagic copepods and (2) if it is, McLaren and Corkett may be correct in arguing that simple body mass (including fat and wax, calcium carbonate of shells, etc.) would be a poor estimator (independent variable) for a predictive equation. For the time being, however, McLaren and Corkett (1984) cannot show for their example, *Calanus glacialis*, whether their approach or the shortcut by Tremblay and Roff (1983) yields the more accurate annual production estimate.

In conclusion, I suggest that rules relating production to biomass are most suitable for making estimates of production for planning field research or for developing the theory of food web relations — K. Banse, *School of Oceanography WB-10, University of Washington, Seattle, WA 98195*.

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BOOK REVIEWS/COMPTES RENDUS

Identification of Larval Fishes of the Great Lakes Basin with Emphasis on the Lake Michigan Drainage. *Edited by Nancy A. Auer. Great Lakes Fishery Commission, Ann Arbor, MI 48105. Special Publication 82-3. 744 p. unbound. $10.50 (U.S.); $12.50 (Canada).*

My own research interests have centered in part on the identification of marine fish larvae and I looked forward to reviewing this publication. I am pleased to say that I found this publication to be very good and provided me with a lot of information on the identification of freshwater early life history stages. The book has a wealth of information, especially detailed descriptions of early life stages. The objective as stated by the editor was "to prepare a concise, descriptive manual for the separation and identification of larval fishes from Lake Michigan and its drainages." This objective has been achieved.

The introduction briefly describes the study area and provides two maps, one of limited value of the Great Lakes watershed and one of the Lake Michigan watershed with the streams and rivers labeled (although difficult to read) and underlaid with the counties (also difficult to read). Several short lists of species are given, including a list of those endangered or threatened. No reason is given for this latter list and it seems out of place in a book of this type. An explanation of the format of the book is provided including a definition of terms. Many of the definitions are too simplified to be of any value.

Dichotomous keys to families are provided for yolk-sac larvae and larvae. I did not have any material to attempt to run through these keys to see if they work. I have never found keys to be particularly useful for identifying larvae for a number of reasons. Among the reasons, for example, is the difficulty in making myomere counts when oftentimes specimens are bent and twisted. To decide if a specimen has more or fewer than 30 myomeres in order to make a decision in key can be difficult unless one has a large series of specimens in excellent condition. It is sort of a "Catch 22" situation to use a key. If you know larvae, you can narrow down its identity from your basic knowledge. If you do not know larvae, you will probably make so many interpretive mistakes in using the key that you will be led hopelessly astray. That is not to say that keys are not of value in some cases. I just question the value of long keys (27 couplets for yolk-sac larvae and 32 for larvae) for identifying larvae to the family level.

The bulk of the book comprises species accounts, and this is where the real value of the book lies. Again, I have no familiarity with freshwater larvae, but these accounts are excellent in their compilation of data and illustrations. The accounts are arranged in family groups with general information followed in some cases by keys to genera and species, which may be useful since the keys are of manageable length. For each species a standard format of information is given on distribution, spawning season, spawning temperature, spawning habitat, spawning substrate, fecundity, natural hybrids, eggs, yolk-sac larvae, larvae, juveniles, adults, and literature cited (author and year only, as full citations are combined at the end of the book). The emphasis is on descriptive characters of the early life history stages. Information is provided on morphometry, morphology, and pigmentation. For adults, meristics and diagnostic features are provided. Illustrations are provided and for the most part these are outstanding, especially those done by Auer and those from papers by Fuiman. The illustrations are very uneven and most of those not done by Auer or Fuiman are of marginal use. It is unfortunate that Auer could not have redrawn all of the poor illustrations because in many of them one cannot distinguish between pigment and structure. The style of printing of the book is not the type that produces halftones satisfactorily. The book concludes with a literature cited section and an index to the scientific names, genus and species only.

The book is not bound; rather it is three-hold punched and without bindings for three reasons as stated by the publisher: to permit binding according to the purchaser's needs, to reduce the purchase price, and to reduce distribution costs. This is certainly admirable today when book costs are generally out of reach for individual scientists.

The book will be of value to all North American freshwater biologists interested in early life histories of fish. It also provides a good overview of ELH stages for most of the families of freshwater fish occurring in North America.

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(R511)

Global Fisheries: Perspectives for the 1980s. *Edited by B. J. Rothschild. Springer-Verlag, New York Inc. 289 p. 1983. 11 fig. $34.80 (U.S.).*

Fisheries, around the globe, are experiencing rough times. Canada is no exception. Few readers will be unaware this is so, but many who are not directly involved with the major fisheries may be wondering just exactly how such a mess could have happened. After all, the global trend to extended fisheries jurisdiction was supposed to have the opposite effect, wasn't it?

This is a book about what went wrong, the major reasons why, and some tentative suggestions for resolving the mess. As multi-authored books go, it is a useful effort. The "great men" invited to contribute have done much better than the usual superficialities dashed off in some airport lounge. For the most part, they have provided thoughtful and clearly written analyses that are worth reading.

Who should read it? There is something for nearly anyone with an interest in fisheries. Certainly the fisheries professionals who have been close to the problem will gain the least, but even

for these there are some interesting morsels they will not have seen in print elsewhere. But given the impressive list of authors, these professionals are likely to read the book regardless of my opinion.

The potential audience is much wider than just the established fishery specialists. There are enough gaps, repetitions, and misplaced emphases that the book could not be used directly in a course, but I think it would serve well in providing students with a sense of the bewildering complexities encountered in managing major fisheries. If I were teaching a fisheries course, I would consider recommending this book as background reading, prior to introducing, for example, the more explicit issues raised in the Pearse and Kirby Commission reports.

By the same reasoning, any reader of this journal who has not been directly involved, but would like an overview of what the fisheries fuss comprises, would find the book rewarding. And, if there remains a fisheries scientist, anywhere, who is yet to be disabused of the notion that fisheries management is primarily an exercise in applied biology, I can only hope that Rothschild's efforts will not fall on stony ground.

That about exhausts the total potential audience that is likely to see this review, but it does not exhaust the list of those who might read it with profit. If I may indulge in an apparent acerbity, some of the major Canadian banks should consider providing copies for their industrial loan departments, in the hope that some of the subtleties of resource exploitation might in future be better appreciated by those who figure so importantly in the persistent overcapitalization and attendant problems we see around us. (Greed is not a nice word, so I won't mention it.)

Taken together, enough has been said to indicate that I think this book is worth reading, and that it should interest a relatively high proportion of readers of this journal. There is a huge literature on the problems facing global fisheries, but if you are looking for one book to give an overview, preferably one that can be read with ease and interest, in front of the fireplace after a tough day at work, this one is a good choice. You won't be exposed to every complexity of this bewildering fishery business, but you will at least be briefly exposed to most of them.

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(R526)

BOOKS RECEIVED/LIVRES REÇUS

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Freshwater Crayfish. V. *Edited by Charles R. Goldman. AVI Publishing Company, Inc., Westport, CT. 1983. xxxvii + 569 p. $35.00 (U.S.). ISBN 0-87055-438-7.* This volume comprises the proceedings of the Fifth Internation Symposium on freshwater crayfish and covers population studies, production, behavior, diseases and parasites, toxic effects of biocides, effects of acidification, and calcium ion accumulation. Production problems including feeds, forage growing locations, trapping techniques, commercialization, and research efforts around the world are also discussed. [R564]

The Biology of Crustacea, Environmental Adaptions. Volume 8. *Edited by F. John Vernberg and Winona B. Vernberg. Academic Press Canada, Don Mills, Ont. 1983. xxvi + 383 p. $67.00 (Canada). IXBN 0-12-106408-5.* In this book the dynamic interaction between an organism and its environment is examined, with emphasis on adaptive significance of its responses. Respiratory adaptions, ventilation, circulation and oxygen transport, feeding mechanisms, chemical and osmotic regulation, reproduction, and adaptations to land and freshwater are treated. [R566]

Key Papers on Fish Populations. *Edited by D. H. Cushing (Ed.). IRL Press Ltd., Oxford, England. 1983. 405 p. £21.00; $42.00 (U.S.). ISBN 0-904-147-58-4.* The book comprises 14 papers, 5 of which provide the basis for fisheries science and the rest represent developments since the 1950's, including current controversies. [R567]

Communication and Behavior of Whales. *Edited by Roger Payne. Westview Press, Boulder, CO. 1984. xii + 643 p. $35.00 (U.S.). ISBN 0-86531-722-4.* One of the AAAS Selected Symposia Series, the book features 14 papers presented at the January 1980 meeting, including vocalization, behavior on breeding grounds, migration and stock identification, research techniques, and an annotated bibliography spanning 1864–1980. [R568]

Chironomidae of the Holarctic Region. Keys and Diagnoses. Part 1. Larvae. *Edited by Torgny Wiederholm. Entomol. Scand. Suppl. 19: 457 p. Lund, Sweden. 1983. $73.00 (U.S.) plus $2 handling charge per order. ISSN 0105-3574. Order from Scandinavian Entomology Co., P.O. Box 24, S-240 17 S. Sandby, Sweden.* Genera of eight subfamilies are described briefly. Comments, along with notes on ecology and distribution, are included in the book, which is intended for use by ecologists. [R569]

A Review of the Genera *Doithrix* n. gen., *Georthocladius* Strenzke, *Parachaetocladius* Walker and *Pseudorthocladius* Goetghebuer (Diptera: Chironomidae, Orthocladiinae). *Edited by Ole A. Saether and James E. Sublette. Entomol. Scand. Suppl. 20: 99 p. Lund, Sweden. 1983. $19.00 (U.S.) plus $2 handling charge per order. ISSN 0105-3574. Order from Scandinavian Entomology Co., P.O. Box 24, S-240 17 S. Sandby, Sweden.* The supplement presents descriptions and keys to males, pupae, and larvae of the genera and subgenera, including similar genera. [R570]

A Catalogue of Chironomid Genera and Subgenera of the World Including Synonyms (Diptera: Chironomidae). Suppl. No. 17. *Edited by Patrick Ashe. Entomol. Scand. Suppl. 17: 68 p. Lund, Sweden. 1983. $15.00 (U.S.) plus $2 handling charge per order. ISSN 0105-3574. Order from Scandinavian Entomology Co., P.O. Box 24, S-240 17 S. Sandby, Sweden.* This catalogue brings together, for the first time, basic information on all chironomid genus-group names, e.g. author, year of publication, type species, etc., described between 1800 and 1983. The catalogue contains 524 recent genus-group names, of which 355 are valid and 169 are either synonyms, nomina dubia, or nomina nuda. In addition, 23 fossil genera are included. [R571]

Amphibians and Reptiles of New England: Habitats and Natural History. *Edited by Richard M. DeGraaf and Deborah D. Rudis. The University of Massachusetts Press, Amherst, MA. 1983. 112 p. $14.00 (U.S.) (cloth) ISBN 0-87023-3998-8; $6.95 (U.S.) (paper) ISBN 0-87023-400-5.* The book is a guide to the natural history and habitats of the inland amphibians and reptiles of New England. It brings together information on the range, relative abundance in New England, habitat, key habitat requirements, breeding period, growth and development, home range and movements, food habits, and key references of each species. The book is not a field guide for species identification. [R572]

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